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(54) Title: MATRIX GENE EXPRESSION IN CHONDROGENESIS

(57) Abstract: The present invention is directed to polypeptides which can be used to stimulate cell growth and/or division. More particularly, the present invention is directed to polypeptides which can be used to stimulate mesenchymal cell growth and/or division. The invention also relates to a method for transfecting chondrocytes and other mesenchymal cells with vectors carrying genes capable of stimulating chondrogenesis, osteogenesis, growth, repair, regeneration and/or restoration of the cellular matrix.



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MATRIX GENE EXPRESSION IN CHONDROGENESIS

Technical Field:

The present invention relates to polypeptides which stimulate cell growth and/or division. More particularly, the present invention relates to polypeptides which stimulate mesenchymal cell growth and/or division. The invention also relates to a method for transfecting chondrocytes and other mesenchymal cells with vectors carrying genes capable of stimulating chondrogenesis, osteogenesis, growth, repair, regeneration and/or restoration of the extracellular matrix.

Background Art:

In the developing embryo, chondrogenesis commences with the proliferation, migration and condensation of mesenchymal stem cells into zones which are destined to become specific regions of the skeleton. This morphogenetic phase is followed by differentiation of mesenchymal-derived cells and the expression by these differentiated cells of matrix proteins characteristic of the tissues they occupy (Figure 1). In the growing limb bud, chondrocytes become the predominant cell type and at a specific stage selectively express genes required to form a cartilaginous matrix. The most abundant matrix gene produced in cartilage is type II collagen (Cancedda et al. 1995; Sandell et al. 1991; Muratoglu et al. 1995) which is co-expressed with the large aggregating proteoglycan, aggrecan. The cartilaginous anlage produced by these cells during chondrogenesis is eventually transformed into the long bones of foetal and post-foetal life by a process of endochondral ossification. This involves the progressive proliferation, maturation, hypertrophy and apoptosis of chondrocytes followed by mineralisation of the lacunae vacated by the chondrocyte, vascular invasion and proliferation of osteoblasts and the deposition of a bone matrix (Figure 2). The bone lengthens longitudinally by the progressive proliferation of chondrocytes followed by the replacement of cartilage by vascularised bone. In the late stages of foetal development and after birth this process takes place in the growth plate where calcification, chondrocyte death, osteoblastogenesis and vascular invasion lead to the formation of bone trabeculae at the interface between the hypertrophic/dying chondrocytes of the cartilage,

All these important cellular events are tightly regulated by genomic, paracrine, autocrine, endocrine and mechanical factors. The identity and the respective roles these factors perform, however, are still largely unknown. Type II collagen is the major structural protein of the cartilage matrix representing approximately 50% of the dry weight of the tissues. This collagen provides the structural scaffold of the matrix, maintaining the overall shape of the cartilage and entrapping the macromolecular hydrated proteoglycan aggregate (aggrecan) within its network. Type II collagen also undergoes ionic, hydrophobic and hydrogen bonding with other matrix molecules such as type IX collagen, fibronectin, osteonectin, hyaluronan and the dermatan sulphate containing proteoglycans, decorin and biglycan. The proteoglycan aggregate, aggrecan because of its high anionic charge and water binding capacity confer the resilience and viscoelastic properties to the tissue necessary for its mechanical functions. The relative distribution of proteoglycans and type II collagen in human foetal cartilage at sites of endochondral ossification as well as the formation of bone are shown in Figure 2.

In contrast to all other species, the antlers of the deer family undergo an annual shedding and regeneration throughout their adult life. The process of antler formation requires the rapid seasonal growth of cartilage from periosteal tissues on the pedicles of the skull with the progressive transformation of the cartilage to bone via endochondral ossification in the distal regions and endochondral ossification and membranous bone formation at the proximal margins (Banks and Newbrey, 1983; Goss, 1983; Kierdorf et al. 1995). The rates of cartilage growth and ossification are unparalleled in the adult vertebrate kingdom (up to 2cm/week). While there are many morphological and histological similarities between the processes of cartilage conversion to bone in the antler and the epiphyseal growth plate (Banks and Newbrey, 1983; Goss, 1983; Kierdorf et al. 1995), there are also differences, particularly in the distal cartilage region which exhibits characteristics of the early stage of cartilage formation (chondrogenesis) *in utero* (Figure 2) (Banks and Newbrey, 1983; Goss, 1983; Kierdorf et al. 1995; Price et al. 1996). In the distal region of the developing antler (Figures 3 and 4) the columbic assembly of chondrocytes is more diffuse than in the epiphyseal growth plate and the non-mineralised cartilaginous zone may be sub-divided morphologically into an outermost tip of mesenchymal cell zone

which merges into a prechondroblastic zone which is penetrated by blood vessels. In the chondroblastic zone located proximally to the prechondroblastic region the cells show typical chondrocyte morphology but with a hypertrophic appearance in the deeper regions (Figure 4). This zone is also served by vascular channels but the extracellular matrix still stains strongly for type II collagen and proteoglycans (Figure 4) which are characteristic gene products of hyaline cartilage. In addition to type II collagen, type I and type III collagens, which are absent from normal growth plate cartilage are reported to be present in the cartilaginous tip of antler (Newbrey et al. 1983).

The two alternatively spliced gene transcripts, type IIA procollagen and IIB are also expressed in the cartilaginous tip of regenerating antler (Price et al. 1996). However, only type IIA procollagen, the isoform which is considered to induce chondrogenesis, was transiently expressed in the chondroprogenitor region of cartilage (Price et al. 1996). Collectively, these reports suggest that the process of chondrogenesis and ossification in the developing deer antler resembles more closely the pattern of long bone formation in early foetal tissue rather than in post-foetal cartilage; however it may be considered as a hybrid of the two.

Degenerative and traumatic injuries to cartilage and other weight bearing connective tissues such as the intervertebral disc, meniscus and tendon are very common but often difficult to treat medically. For example, the injury to diarthrodial joints can be sufficiently intense as to cause chondral or osteochondral fractures, while disc and tendon rupture leads to cell necrosis, neurological and vascular deficits, which apart from impairment of function are accompanied by long-term morbidity. If the injury to connective tissues such as joint cartilage penetrates into the subchondral bone (osteochondral defects) imperfect healing in the form of fibrocartilage formation can occur. This type of repair is mechanically inferior to the original tissue and can fail under everyday stress loading. When the injury to the connective tissue is confined to the avascular regions, healing rarely occurs spontaneously (Buckwalter et al. 1987). The process of cartilage, disc or other connective tissue injury may also be exacerbated in older subjects where cell numbers and their viability and ability to respond to growth factors may already be diminished (Löeser et al. , 2000; Hashimoto et al. 1998). It is common in these instances that more progressive cartilage or

disc degeneration follows leading to overload of subchondral bone in adjacent supporting structures and the onset of osteoarthritis (OA). Thus it is generally agreed by those skilled in the art that it is the avascularity and the end stage differentiation of connective tissue cells which precludes their normal
5 regeneration and repair following injury. Furthermore, this situation is exacerbated by the low density, diminished responsiveness and viability of cells within the connective tissues resulting from the aging process, altered hormonal status, mechanical factors and impaired nutrition. These deficiencies are of considerable significance since failure of connective tissue
10 function, as occurs with OA of the peripheral joints and spine, are the most frequent cause of pain and disability in all societies and represents the most common rheumatic disorder worldwide.

Within recent years, attempts have been made to promote tissue regeneration and repair in cartilage, meniscus, tendon and the intervertebral
15 disc by a variety of methods. Some of the approaches employed are described in recent publications on this subject (Buckwalter and Mankin, 1998; Breinan et al. 1998; Wakitani et al. 1998, Rahfoth et al. 1998; Nishida et al. 2000; Moon et al. 2000). In the Buckwalter and Mankin (1998) article the authors conclude that "None of the current procedures for repairing or
20 transplanting articular cartilage restores a normal articular surface, but they can decrease symptoms associated with chondral defects in some patients". A common method for undertaking cartilage repair is to use autologous transplantation of chondrocytes (supported by an artificial matrix) into the chondral defects. Clinical reports suggest that this surgery is effective in
25 repairing small defects in younger patients (Brittberg et al. 1994; Peterson, 1996) but the procedure is still far from satisfactory due to the inherent limited proliferative and biosynthetic capacity of the mature chondrocyte for the reasons already cited. As discussed, attempts to overcome this problem by breaching the subchondral plate by drilling or fenestration to allow
30 undifferentiated mesenchymal cells of the bone marrow to penetrate and occupy the defect have also only been partially successful. The material that initially occupies these defects invariably deteriorates to fibrocartilage which, by its very nature, is incapable of performing the specialised biomechanical functions required of articular cartilage (Nehrer et al. 1999). Nehrer and co-
35 workers (1999) showed that cells which repaired a chondral defect in rabbit joints expressed low transcription levels of the type II collagen gene due to

insufficient differentiation of mesenchymal cells of the bone marrow to the chondrocyte phenotype. From these findings they conclude that it was the absence of sufficient amounts of fundamentally important regulatory factors, or progenitor cells, in the repair tissue which inhibited its transformation to normal cartilage.

In more recent years attempts to overcome some of these problems has led to the utilisation of the technique of transfecting connective tissue cells grown in a compatible biomatrix with growth factor genes or other genes which could promote regeneration or decrease turnover of the extracellular matrix. Examples of this approach include: Cultured equine articular chondrocytes, mesenchymal stem cells, synovial explants, and synovial intimal cells were transfected with an E1-deleted adenoviral vector containing equine insulin-like growth factor-I coding sequence. (Nixon et al. 2000). Discs injected with Ad/CMV-hTGF β 1 exhibited extensive and intense positive immunostaining for transforming growth factor β 1 with the nucleus pulposus showing a 30-fold increase in active transforming growth factor β 1 production. Furthermore, tissues so transfected synthesised 100 % more proteoglycan relative to non transfected control tissue (Nishida et al. 1999). The use of gene transfer of antiinflammatory cytokines or the *in vivo* induction of their expression has been described as a potential method for the treatment of osteoarthritis by decreasing matrix degradation (Fernandes et al. 2000). Others researchers have used monolayer cultures of bovine chondrocytes seeded onto polylactic acid (PLA), polyglycolic acid (PGA), collagen matrices to induce the production of collagen type I, collagen type II, and aggrecan. The collagen type I gene was upregulated on collagen scaffolds throughout the culture period but PLA and PGA showed initial induction followed by downregulation (Saldanha and Grande, 2000). Bone morphogenic protein-7 is a member of a family of 16 related BMPs of the TGF- β superfamily. While the major site of action of BMPs is thought to be bone, it has also been shown to have effectiveness in cartilage repair by stimulating synthesis of type II collagen and aggrecan in human articular chondrocytes when administered as a gene-enhanced tissue within a biomatrix into the defects (Mason et al. 2000).

It is clear from the existing art that repair of defects within avascular connective tissue has been largely confined to the transplantation into the defect of biomatrices seeded with host cells transfected with growth factor or

cytokine/anti-cytokine genes which are normally expressed by the those cells but at a reduced level in their non-transfected state. The rational for such an approach is that the amount of extracellular matrix synthesised by the cell will be increased in the transfected cells thereby filling the defect and
5 supporting repair or alternatively transfecting them with genes which diminish the rate at which the matrix produced by the cell is catabolised. While such approaches may provided some benefit, none have exploited the inherent genetic information for growth, repair, regeneration and/or restoration which already exists within the target cells and which was once
10 expressed during foetal development and growth but, because of the advanced state of differentiation and maturation of those cells, may no longer be expressed.

Disclosure of Invention:

15 The present inventors have identified polypeptides that are expressed in high levels in growing/dividing cells. Accordingly, the present invention provides for the use of these polypeptides in stimulating cell growth and/or division.

Thus, in a first aspect, the present invention provides a method of
20 stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:1,
- b) a sequence as shown in SEQ ID NO:2,
- 25 c) a sequence as shown in SEQ ID NO:3, and
- d) a sequence which is at least 50% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 60%, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least
30 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- 35 a) a sequence as shown in SEQ ID NO:4,
- b) a sequence as shown in SEQ ID NO:5,

c) a sequence as shown in SEQ ID NO:6, and

d) a sequence which is at least 70% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more
5 preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected
10 from the group consisting of:

a) a sequence as shown in SEQ ID NO:7,

b) a sequence as shown in SEQ ID NO:8,

c) a sequence as shown in SEQ ID NO:9, and

d) a sequence which is at least 80% identical to any one of (a) to (c).

15 In a preferred embodiment, the polypeptide is at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or
20 inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

a) a sequence as shown in SEQ ID NO:10,

b) a sequence as shown in SEQ ID NO:11,

c) a sequence as shown in SEQ ID NO:12, and

25 d) a sequence which is at least 85% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of
30 stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

a) a sequence as shown in SEQ ID NO:13,

b) a sequence as shown in SEQ ID NO:14, and

35 c) a sequence which is at least 70% identical to any one of (a) or (b).

In a preferred embodiment, the polypeptide is at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) or (b).

5 In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:15,
- 10 b) a sequence as shown in SEQ ID NO:16, and
- c) a sequence which is at least 50% identical to any one of (a) or (b).

In a preferred embodiment, the polypeptide is at least 60%, more preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) or (b).

15 In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- 20 a) a sequence as shown in SEQ ID NO:17, and
- b) a sequence which is at least 60% identical to a).

In a preferred embodiment, the polypeptide is at least 70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to a).

25 In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- 30 a) a sequence as shown in SEQ ID NO:18,
- b) a sequence as shown in SEQ ID NO:19,
- c) a sequence as shown in SEQ ID NO:20, and
- d) a sequence which is at least 50% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 60%, more preferably at least 70%, more preferably at least 80%, even more preferably at

35

least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or
5 inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:21,
- b) a sequence as shown in SEQ ID NO:22,
- c) a sequence as shown in SEQ ID NO:23, and
- 10 d) a sequence which is at least 65% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

15 In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:

- x) a) a sequence as shown in SEQ ID NO:24,
- 20 b) a sequence as shown in SEQ ID NO:25,
- c) a sequence as shown in SEQ ID NO:26, and
- d) a sequence which is at least 75% identical to any one of (a) to (c).

In a preferred embodiment, the polypeptide is at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more
25 preferably at least 97%, and most preferably at least 99% identical to any one of (a) to (c).

In another aspect, the present invention provides a method of stimulating cell growth and/or division, the method comprising contacting, or
inserting into, an animal cell a polypeptide comprising a sequence selected
30 from the group consisting of:

- a) a sequence as shown in SEQ ID NO:27, and
- b) a sequence which is at least 35% identical to a).

The increased cell division and/or matrix gene expression by chondrogenesis may result from the action of transthyretin.

35 In a preferred embodiment, the polypeptide is at least 40%, more preferably at least 50%, more preferably at least 60%, more preferably at least

70%, more preferably at least 80%, even more preferably at least 90%, even more preferably at least 95%, even more preferably at least 97%, and most preferably at least 99% identical to (a).

5 In a preferred embodiment of all previous aspects, the cell is a somatic cell. More preferably, the somatic cell is a mesenchymal cell. More preferably, the mesenchymal cell is selected from the group consisting of: chondrocytes and osteocytes.

10 In a preferred embodiment of all previous aspects, the polypeptide is provided by introducing into the cell an expression vector encoding the polypeptide.

In a further preferred embodiment of all previous aspects, the cell is removed from an animal, preferably a mammal, cultured *in vitro*, transformed or transfected with a polynucleotide encoding the polypeptide and then placed back into an animal.

15 In this regard, and in a particularly preferred embodiment, the present invention provides a method of stimulating chondrogenesis, cartilage, disc or connective tissue growth, repair, regeneration and/or restoration in an animal, the method comprising transfecting a chondrocyte or other mesenchymal cell from an animal with a polynucleotide encoding the polypeptide, and
20 transplanting said transformed chondrocyte or other mesenchymal cell into the animal at a suitable site such that, at said site, the polynucleotide molecule is expressed in the chondrocyte or other mesenchymal cell thereby causing chondrogenesis, cartilage, disc or connective tissue growth, repair, regeneration and/or restoration in the animal.

25 The cell may be removed from the animal (e.g. a human), transfected and then placed in the animal, preferably at the site where chondrogenesis, cartilage, disc or connective tissue growth, repair, regeneration and/or restoration is required in the animal.

One example of this embodiment comprises the use of a 1.5 kb full
30 length cDNA prepared from clone DACC-7 according to standard techniques which is cloned into a vector such as pBK-CMV.2 (as described herein) and transfected into chondrocytes according to the method described by Goomer et al. (2000) where it was observed that lapine chondrocytes grown in pellet culture showed enhanced proliferation as determined by the higher
35 incorporation of the radioactive precursor, ³H-thymidine, into DNA produced by these cells (Figure 6). These pellet culture keep the chondrocyte

phenotype as shown by Goomer et al. (2000) even though they are proliferating.

In a further preferred embodiment of all previous aspects, the cell is transformed or transfected *in vivo* with a polynucleotide encoding the polypeptide.

In this regard, and in a particularly preferred embodiment, the present invention provides a method of stimulating chondrogenesis, cartilage, disc or connective tissue growth, repair, regeneration and/or restoration in an animal, the method comprising transfecting *in vivo* a chondrocyte or other mesenchymal cell in an animal (see US Patent 6,159,464 and Goomer et al. 2000) with a polynucleotide encoding the polypeptide, such that the polynucleotide molecule is expressed in the chondrocyte or other mesenchymal cell thereby causing chondrogenesis, cartilage, disc or connective tissue growth, repair, regeneration and/or restoration in the animal.

In another aspect, the present invention provides a method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide as outlined in the previous aspects.

In another aspect, the present invention provides a method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- i) exposing the polypeptide to a candidate agent, and
 - ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
- wherein the polypeptide is a polypeptide as outlined in the previous aspects.

In one embodiment, the agent inhibits the ability of the polypeptide to stimulate cell growth and/or division.

In another embodiment, the agent enhances the ability of the polypeptide to stimulate cell growth and/or division.

In a particularly preferred embodiment of all previous aspects, the animal cell is a mammalian cell. More preferably, the mammalian cell is a human cell.

In a further aspect, the present invention provides a method of stimulating mesenchymal cell growth and/or division, the method comprising

exposing animal mesenchymal cells to conditioned media, or an active fraction thereof, obtained from deer antler cartilage cells.

The conditioned media can be obtained from any culture in which deer antler cartilage cells are grown *in vitro*. One example, as exemplified herein
5 is growing the deer antler cartilage cells in DMEM:F12/10%(v)FBS.

As used herein, the term "active fractions thereof" refers to at least partially purified portions of the conditioned media that maintain the factor(s) which stimulate mesenchymal cell growth and/or division.

Preferably, the deer antler cartilage cells are selected from the group
10 consisting of: prechondrocytes, mature chondrocytes, hypertrophic chondrocytes, or a combination thereof.

Preferably, the method further comprises exposing the cells to a growth factor. More preferably, the growth factor is selected from the group consisting of: insulin-like growth factor (IGF-1), TGF-beta, fibroblast growth
15 factor (FGF), vascular endothelial growth factor (VEGF), morphogenic bone factors, thyroid hormones (thyroxine), parathyroid hormone related protein (PTHrP), sex hormones, luteinizing hormone (LH) and prolactin.

The present inventors unexpectedly determined that chondrocytes of rapidly growing cartilage of regenerating deer antler express unique genes
20 which are not expressed in mature articular cartilage chondrocytes or chondrocytes of the epiphyseal growth plate as observed on Northern Blot analysis of deer chondrocyte mRNA. Of even greater surprise was the finding that some of these gene transcripts are also expressed in the early stage of chondrogenesis in the human foetal tissues as demonstrated by *in-situ*
25 hybridisation (the results of which are provided hereinafter).

Accordingly, in another aspect the present invention provides an isolated polynucleotide molecule comprising a nucleotide sequence encoding a gene product expressed in chondrocytes of rapidly growing cartilage of regenerating deer antler.

30 Preferably, the novel gene product is one which is also expressed in the early stage of chondrogenesis in human foetal tissue and in human chondrocytes and like cells attempting to restore the extracellular matrix and thus functionality of degenerate and osteoarthritic cartilages.

In a further aspect, the present invention provides a substantially
35 purified polypeptide comprising a sequence selected from the group consisting of:

a) a sequence as shown in SEQ ID NO:1, and

b) a sequence which is at least 91% identical to a),

wherein the polypeptide is capable of stimulating animal cell growth and/or division.

5 Preferably, the polypeptide is at least 95% identical to a). More preferably, the polypeptide is at least 99% identical to a).

 In another aspect, the present invention provides a substantially purified polypeptide comprising a sequence selected from the group consisting of:

10 a) a sequence as shown in SEQ ID NO:4, and

b) a sequence which is at least 99% identical to a),

wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or a structural component of extracellular matrix.

15 In another aspect, the present invention provides a substantially purified polypeptide comprising a sequence selected from the group consisting of:

a) a sequence as shown in SEQ ID NO:7, and

b) a sequence which is at least 99% identical to a),

20 wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or a subunit involved in protein synthesis.

 In another aspect, the present invention provides a substantially purified polypeptide comprising a sequence selected from the group

25 consisting of:

a) a sequence as shown in SEQ ID NO:13, and

b) a sequence which is at least 90% identical to a),

wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or altering

30 chromatin structure.

 Preferably, the polypeptide is at least 95% identical to a). More preferably, the polypeptide is at least 99% identical to a).

 In another aspect, the present invention provides a substantially purified polypeptide comprising a sequence selected from the group

35 consisting of:

a) a sequence as shown in SEQ ID NO:15, and

b) a sequence which is at least 99% identical to a),
wherein the polypeptide has a biological activity selected from the group
consisting of: stimulating animal cell growth and/or division, or regulating
cell migration.

5 In another aspect, the present invention provides a substantially
purified polypeptide comprising a sequence selected from the group
consisting of:

a) a sequence as shown in SEQ ID NO:18, and

b) a sequence which is at least 91% identical to a),

10 wherein the polypeptide has a biological activity selected from the group
consisting of: stimulating animal cell growth and/or division, or responses to
cell stress.

Preferably, the polypeptide is at least 95% identical to a). More
preferably, the polypeptide is at least 99% identical to a).

15 In another aspect, the present invention provides a substantially
purified polypeptide comprising a sequence selected from the group
consisting of:

a) a sequence as shown in SEQ ID NO:21, and

b) a sequence which is at least 96% identical to a),

20 wherein the polypeptide has a biological activity selected from the group
consisting of: stimulating animal cell growth and/or division, or a component
of connective tissue, or collagen fibrillogenesis.

Preferably, the polypeptide is at least 99% identical to a).

In another aspect, the present invention provides a substantially
25 purified polypeptide comprising a sequence selected from the group
consisting of:

a) a sequence as shown in SEQ ID NO:24, and

b) a sequence which is at least 98% identical to a),

30 wherein the polypeptide has a biological activity selected from the group
consisting of: stimulating animal cell growth and/or division, or a component
of collagen.

Preferably, the polypeptide is at least 99% identical to a).

The present invention also provides the deer ortholog of human
transthyretin (SEQ ID NO:27) which comprises the sequences
35 FVEGL/IYQ/KVEL/IDTK (SEQ ID NO: 41) and EGL/IYQ/KV (SEQ ID NO: 42).

In another aspect, the present invention provides a fusion protein comprising a polypeptide according to the present invention.

In a preferred embodiment of this aspect, the at least one other polypeptide is selected from the group consisting of: a polypeptide that
5 enhances the stability of the polypeptide of the present invention, and a polypeptide that assists in the purification of the fusion protein.

In a further aspect, the present invention provides an isolated polynucleotide encoding a polypeptide according of the present invention.

Preferably, the polynucleotide comprises a sequence according to any
10 one of SEQ ID NO:28, 29, 31 to 33, or 35 to 38.

In yet another aspect, the present invention provides an isolated polynucleotide comprising a sequence provided as SEQ ID NO:30.

In another aspect, the present invention provides an isolated polynucleotide comprising a sequence provided as SEQ ID NO:34.

15 In a further aspect, the present invention provides an antisense polynucleotide which hybridizes under high stringency conditions to a polynucleotide of the present invention.

In a further aspect, the present invention provides a vector comprising the polynucleotide according to the present invention.

20 Preferably, the polynucleotide is operably linked to a promoter.

The vectors may be nonviral (synthetic) or viral, as well as plasmid, or phage vectors provided with an origin of replication, and preferably a promoter for the expression of the polynucleotide molecule and, optionally, a regulator of the promoter. The vector may contain one or more selectable
25 markers, for example, an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for an animal expression vector. Other selectable markers may be used in accordance with the application at hand. The vector may be used *in vitro*, for example, for the production of RNA or used to transfect or transform a host cell.

30 In another aspect, the present invention provides a host cell transfected or transformed with a vector according to the present invention.

Preferably, the host cell is an animal cell. More preferably, the host cell is a mammalian cell.

In a further aspect, the invention provides a method of identifying
35 and/or characterising the developmental position of mesenchymal cells, particularly during embryogenesis, the method comprising exposing a test

sample including mesenchymal cell mRNA to a suitably-labelled nucleic acid probe with specifically hybridizes to a polynucleotide of the present invention and detecting hybridisation of said probe to said mRNA.

Preferably, the test sample is a suitably prepared histological section.

- 5 In a further aspect, the present invention provides antibodies which specifically bind to a polypeptide of the present invention, as well as the use of the antibodies to block the ability of the polypeptide to stimulate cell growth and/or division.

Throughout this specification, unless the context requires otherwise, 10 the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood, 15 preferred forms will be described with reference to the following examples and drawings.

Brief Description of Figures:

Figure 1 shows a diagrammatic representation of endochondral bone 20 formation in the foetus. In the early stages of embryogenesis, mesenchymal cells in the limb bud condense (A - C) to form a cartilaginous anlage (D). In the diaphysis of the anlage, chondrocytes hypertrophy and a boundary is formed between them and the surrounding undifferentiated stacked cells (E). Blood vessels invade the nondifferentiated cellular region of the anlage (F). A 25 primitive marrow cavity is formed and the remaining cartilage establishes the epiphyseal growth plates (G). Secondary ossification centres arise concomitantly with vascularisation of the epiphysis allowing longitudinal growth (G) and (H) (Adapted from Cancedda et al. 1995).

30 Figure 2 shows histochemical and immunohistochemical staining of proteoglycans and type II collagen in sections of 12-week-old human foetal distal phalanges to demonstrate their respective distribution in the tissues as well as the morphology of the endochondral ossification process. A (x16), D (x50), G (x100) = Masson Trichrome staining of collagens of dermis, 35 connective tissue, blood vessels and blood cells of the foetal joint. B (x16), E (x50), H (x100) = Toluidine Blue staining showing proteoglycan distribution

in the epiphyseal hyaline cartilage. C (x16), F (x50), I (x100) = type II collagen immunostaining of hyaline cartilage complementary to proteoglycan distribution (Toluidine Blue). Note the invasion by blood vessels and resorption of cartilage matrix corresponding to early endochondral ossification of the central metaphysal shaft.

Figure 3 is a diagrammatic representation of the cartilaginous (non-ossified) tip of deer antler showing the three main cellular regions designated as A, B and C corresponding to the PC (prechondrocyte), MC (mature chondrocyte) and HC (hypertrophic chondrocyte) phenotypes respectively. Panel A shows the tissue sampled only included the central cartilage core thereby excluding fibrous periosteum and regions considered to have undergone intramembranous ossification. Panel B shows cells from each of these three regions (A, B, C) were processed separately for cell culture studies and their total RNA extracted; also whole cartilaginous tip sections were used for histological, immunohistochemical, and in situ hybridisation studies, as well as total RNA was extracted from the whole cartilaginous tip.

Figure 4 shows histochemical and immunohistochemical staining of cartilage sections taken from region B (mature chondrocytes) of deer antler cartilage. Panel A (x50), B (x100) = Note Toluidine Blue staining of proteoglycan in cartilage matrix between vascular channels (unstained). Panels C (x50) and D (x100) show immunostaining for type II collagen of region B cartilage which is seen to be complementary to proteoglycan staining with Toluidine Blue (Panels A and B).

Figure 5

- (i) DACC-2. Length: 1.426 kb. Underline indicates 93% homology of a 1.404 kb overlap with Human alpha1 type II collagen cDNA.
- (ii) DACC-3. Length: 0.957 kb. Underline indicates 89% homology of a 0.876 kb overlap with Human ribosomal protein S2 (RPS2) cDNA.
- (iii) DACC-4. Length: 0.532 kb. Underline indicates 92% homology of a 0.486 kb overlap with Human ribosomal protein L23a (RPL23A) cDNA.
- (iv) DACC-5. Length: 1.224 kb. Underline indicates 89% homology of a 1.189 kb overlap with Human non-histone chromosomal protein (HMG-14) cDNA.

(v) DACC-6. Length: 1.163 kb. Underline indicates 91% homology of a 1.145 kb overlap with Human mRNA for KIAA1075 protein (tensin2) cDNA.

(vi) DACC-7. Length: 1.506 kb. Underline indicates 70% homology of a 1.506 kb (entire length) overlap with Human mRNA similar to RIKEN cDNA
5 0610011N22 gene (LOC133957, Genbank BC015349).

(vii) DACC-8. Length: 1.088 kb. Underline indicates 83% homology of a 1.086 kb overlap with Human SPARC/osteonectin cDNA.

(viii a) DACC-9 (5'end). Length: 0.410 kb. Underline indicates 89% homology of a 0.359 kb overlap with Human mRNA similar to HEAT-SHOCK
10 20 KDA LIKE-PROTEIN P20 (LOC126393, Genbank AK056951) and Human sequence 109 from Patent WO9954460 (Genbank AX013767) cDNA.

(viii b) DACC-9 (3'end). Length: 0.588 kb. Underline indicates 79% homology of a 0.584 kb overlap with Human mRNA similar to HEAT-SHOCK
20 KDA LIKE-PROTEIN P20 (LOC126393, Genbank AK056951) and Human
15 sequence 109 from Patent WO9954460 (Genbank AX013767) cDNA.

(ix) DACC-10. Length: 1.625 kb. Underline indicates 90% homology of a 1.578 kb overlap with Human procollagen alpha2(V) cDNA.

(x) DACC-11. Length: 1.508 kb. Underline indicates 95% homology of a 1.508 kb overlap with Human prepro-alpha1(I) collagen cDNA.

20

Figure 6 shows the incorporation of ³H-thymidine (counts per minute/microgram DNA) into DNA synthesised by vector alone (**mock**) and vector with DACC-7 transfected (**DACC-7**) lapine chondrocytes grown in pellet culture as described previously (Goormer et al. 2000). Note the higher
25 incorporation of radioactivity into synthesised DNA of DACC-7 transfected cells.

Figure 7 shows *in situ* hybridisation for DACC-7 mRNA on sections of 12-week-old human foetal knee joints showing expression of this gene product
30 in epiphyseal hyaline cartilage but low expression in the meniscal cells. A (x16), C (x50), D (x100) DACC-7 mRNA, B (x16) = negative control.

Figure 8 shows *in situ* hybridisation for type II collagen mRNA of sections of 12-week-old human foetal knee joints. A (x16), B (buffer only control), C
35 (x50), D (x100). Note expression of type II collagen mRNA in both hyaline epiphyseal cartilage as well as the fibrocartilaginous meniscus (contrast with

immunostaining where no type II collagen protein in meniscus was observed).

Figure 9 shows *in situ* hybridisation for DACC-7 mRNA in sections of 12 and 14-week-old human foetal knee joint epiphyseal cartilage showing decreased expression of message in the 14-week-old relative to 12-week-old specimen. A (x200), C (x400) = 12-week-old joint. B (x200), D (x400) = 14-week-old joint.

Figure 10 shows a photomicrograph of sagittal histological section of the anterior region of a 12-week-old human foetal spinal column. Panel A: Toluidine Blue stained section showing disc and adjacent cartilaginous vertebral bodies, the notochordal cell cluster of the nucleus pulposus (NP) and the alignment of fibrocytes of the annulus fibrosis (AF) (x100). Panel B: Toluidine Blue stained section of disc and adjacent cartilaginous vertebral bodies showing NP and AF at higher magnification (x200). Panel C: Toluidine Blue stained section showing demarcation of cells in the cartilage anlage of the vertebral body and the adjacent fibrous AF (x400). Panel D: Higher power photomicrograph of the NP showing the notochordal cells and cells of the inner AF which will develop into the transitional zone (x400). Panel E: *In situ* hybridisation for DACC-7 expression by cells of the cartilage anlage and the transition to the AF using an antisense probe. Note the stronger staining of chondrocytes than fibrocytes (x400). Panel F: *In situ* hybridisation for DACC-7 expression by cells of the NP using an antisense probe. Note the strong staining of notochordal cells (x400).

Figure 11 shows a photomicrograph of sagittal histological section of the anterior region of a 12-week-old human foetal spinal column. Panel A: *In situ* hybridisation for type II collagen expression by disc cells and the chondrocytes of the cartilage anlage of the vertebral body using a sense probe (x50). Panel B: *In situ* hybridisation for type II collagen expression by disc cells and the chondrocytes of the cartilage anlage of the vertebral body using a antisense probe showing expression in disc cells and cells of the adjacent cartilaginous vertebral bodies (x50). Panel C: *In situ* hybridisation for type II collagen expression by disc cells and the chondrocytes of the cartilage anlage of the vertebral body using a sense probe (x400). Panel D: *In situ*

- hybridisation for type II collagen expression by disc cells and the chondrocytes of the cartilage anlage of the vertebral body using an antisense probe showing demarcation of cells in the cartilage anlage of the vertebral body and the adjacent fibrous AF (x400). Panel E: *In situ* hybridisation for
- 5 DACC-7 expression by the notochordal cell cluster of the nucleus pulposus (NP) using a sense probe (x400). Panel F: *In situ* hybridisation for DACC-7 expression by cells of the NP using an anti-sense probe. Note the strong staining of notochordal cells (x400).
- 10 Figure 12 shows a photomicrograph of coronal histological sections of 14-week-old human foetal finger joint showing articulating surfaces and epiphyseal cartilage. Panel A: Toluidine Blue stained section showing proteoglycan distribution in the extracellular matrix of all cartilages and hypertrophic chondrocytes at the edge of the metaphysis (x50). Panel B:
- 15 Toluidine Blue stained section showing proteoglycan distribution in cartilages of the articulating surfaces and epiphysis (x100). Panel C: *In situ* hybridisation for type II collagen expression by chondrocytes in serial sections of Panel B using a sense probe (x100). Panel D: *In situ* hybridisation for type II collagen expression by chondrocytes in serial sections of Panel B
- 20 using an antisense probe (x100). Panel E: *In situ* hybridisation for DACC-7 expression by chondrocytes in serial sections of Panel B using a sense probe (x100). Panel F: *In situ* hybridisation for DACC-7 expression by chondrocytes in serial sections of Panel B using an antisense probe (x100).
- 25 Figure 13 shows a photomicrograph of sagittal histological section of fragments of degenerate tibial plateau articular cartilage from a human OA joint. Panel A: Toluidine Blue stained section showing distribution of proteoglycans (x200). Panel B: Toluidine Blue stained section showing distribution of proteoglycans (x400). Panel C: *In situ* hybridisation of the OA
- 30 cartilage cells for expression of type II collagen using a sense probe (x200). Panel D: *In situ* hybridisation of the OA cartilage cells for expression of type II collagen using an antisense probe (x200). Panel E: *In situ* hybridisation for DACC-7 expression by chondrocytes in OA cartilage using a sense probe (x200). Panel F: *In situ* hybridisation for DACC-7 expression by chondrocytes
- 35 in OA cartilage using an antisense probe (x200). Panel G: *In situ* hybridisation for DACC-7 expression by chondrocytes in OA cartilage using a

sense probe (x400). Panel H: *In situ* hybridisation for DACC-7 expression by chondrocytes in OA cartilage using an antisense probe (x400).

Figure 14 shows a photomicrograph of horizontal histological sections of region B of fallow deer antler showing mature and hypertrophic chondrocytes assembled in a cartilaginous matrix surrounding the endothelium of vascular channels. Panel A: Toluidine Blue stained section (x200). Panel B: Toluidine Blue stained section (x400). Panel C: *In situ* hybridisation for type II collagen expression by antler chondrocytes using the sense probe (x400). Panel D: *In situ* hybridisation for type II collagen by antler chondrocytes using an antisense probe (x400). Panel E: *In situ* hybridisation for DACC-7 expression by antler chondrocytes using a sense probe (x200). Panel F: *In situ* hybridisation for DACC-7 expression by antler chondrocytes using an antisense probe (x200). Panel G: *In situ* hybridisation for DACC-7 expression by antler chondrocytes using a sense probe (x400). Panel H: *In situ* hybridisation for DACC-7 expression by antler chondrocytes using an antisense probe (x400).

Figure 15 shows the predicted amino acid sequence, size and pI for DACC-7. The amino acid usage, identity and similarity with human (LOC133957) and mouse (RIKEN 0610011N22) homologs of DACC-7 are also shown.

Figure 16 shows the kinetics of stimulation of ^{35}S -PG synthesis in alginate beads of DAC cells from the three antler zones A, B, C. shown in Figure 3. * B > A = C (p < 0.05).

Figure 17 shows the kinetics of DNA synthesis (as ^3H -thymidine incorporation) by DAC cells from zones A, B, C cultured in alginate beads. * B > A = C (p < 0.05).

Figure 18 shows the kinetics of DNA synthesis by ovine articular chondrocytes cultured in the presence of bovine serum albumin (BSA), 10% foetal bovine serum (FBS) or conditioned media from alginate bead cultures of DAC cells from zones A or B from two different animals (2, 3).

Figure 19 shows the kinetics of stimulation of ^{35}S -PG synthesis by ovine femoral condylar chondrocytes incubated for 24 h with various amounts of conditioned media (CM) from alginate bead cultures of DAC cells from zones A (□), B (▨), C (■). *A = B < C (p < 0.05). # = relative to foetal bovine serum (FBS) alone.

Figure 20 shows the kinetics of stimulation of ^{35}S -PG synthesis by ovine tibial plateau chondrocytes incubated for 24 h with various amounts of conditioned media (CM) from alginate bead cultures of DAC cells from zones A (□), B (▨), C (■). *A = B > C (p < 0.05).

Figure 21 shows the kinetics of DNA synthesis (^3H -thymidine incorporation) by ovine chondrocytes incubated for 24 h with various amounts conditioned media (CM) from alginate bead cultures of DAC cells from zones A (□), B (▨), C (■). *A = B > C (p < 0.05). # = relative to foetal bovine serum (FBS) (p < 0.05).

Figure 22 shows the mitochondrial activity in ovine chondrocytes [determined using the MTT assay] after 24 h incubation with various concentrations of conditioned media (CM) from alginate bead cultures of DAC cells from zones A (□), B (▨), C (■). *B = C (p < 0.05). # = relative to FBS (P < 0.05).

Figure 23 shows the kinetics of stimulation of ^{35}S -PG synthesis by ovine condylar chondrocytes incubated with conditioned media (CM) collected for up to 7 days from monolayer cultures of DAC cells (all zone) from different animals (F4, F5, R6.1, R6.2). (■) 1 day, (□) 3 day, (▨) 5 day, (▩) 7 day. # = relative to FBS alone (p < 0.05).

Figure 24 shows the kinetics of stimulation of ^{35}S -PG synthesis by lapine cartilage explants incubated with conditioned media (CM) collected for up to 7 days from monolayer cultures of DAC cells from different animals (F4, F5, R6.1, R6.2). (■) 1 day, (□) 3 day, (▨) 5 day, (▩) 7 day. # = relative to FBS (p < 0.05).

Figure 25 shows the kinetics of DNA synthesis (^3H -thymidine incorporation) by confluent murine 3T3 fibroblasts incubated with conditioned media (CM) from alginate bead cultures from different zones (A), (B), (C) from DAC or monolayer cultures (F4, F5, R6.1, R6.2). # = relative to FBS alone ($p < 0.05$).

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Figure 26 shows a two-dimensional pH 5-8 gradient gel electrophoretogram of concentrated conditioned media from alginate cultures of deer antler chondrocytes which was collected over the first 24 h of culture. The spots circled in red were not present in 7 d cultures of the same cells which corresponded to loss of stimulatory activity of the culture media. Proteins 1 and 2 within these red circles were submitted for Q-TOF MS/MS mass spectrometry. Both proteins were identified as transthyretin on the basis of their partial amino acid sequences.

Figure 27 shows restriction enzymes chosen for construction of a full length DACC-7 cDNA. The restriction enzymes used were EcoRI, SacI and KpnI. These enzymes were chosen on the basis of location in overlapping regions and order of restriction enzyme sites within the multiple cloning region of the plasmid.

20

Key to the Sequence Listing:

SEQ ID NO:1 - Deer polypeptide sequence encoded by DACC-7.

SEQ ID NO:2 - Human polypeptide orthologous to SEQ ID NO:1 (Accession No. XP_059677)

25 SEQ ID NO:3 - Mouse polypeptide orthologous to SEQ ID NO:1 (Accession No. NP_077163).

SEQ ID NO:4 - Deer polypeptide sequence encoded by DACC-2.

SEQ ID NO:5 - Human polypeptide orthologous to SEQ ID NO:4 (Accession No. P02458).

30 SEQ ID NO:6 - Mouse polypeptide orthologous to SEQ ID NO:4 (Accession No. B41182).

SEQ ID NO:7 - Deer polypeptide sequence encoded by DACC-3.

SEQ ID NO:8 - Human polypeptide orthologous to SEQ ID NO:7 (Accession No. P15880).

35 SEQ ID NO:9 - Mouse polypeptide orthologous to SEQ ID NO:7 (Accession No. P25444).

- SEQ ID NO:10 - Deer polypeptide sequence encoded by DACC-4.
SEQ ID NO:11 - Human polypeptide orthologous to SEQ ID NO:10 (Accession No. NP_000975).
SEQ ID NO:12 - Rat polypeptide orthologous to SEQ ID NO:10 (Accession No. CAA46336).
5 SEQ ID NO:13 - Deer polypeptide sequence encoded by DACC-5.
SEQ ID NO:14 - Human polypeptide orthologous to SEQ ID NO:13 (Accession No. XP_049753).
SEQ ID NO:15 - Deer polypeptide sequence encoded by DACC-6.
10 SEQ ID NO:16 - Human polypeptide orthologous to SEQ ID NO:15 (Accession No. XP_029631).
SEQ ID NO:17 - Human polypeptide orthologous to protein encoded by full length cDNA comprising SEQ ID NO:34 (human osteonectin) (Accession No. P09486).
15 SEQ ID NO:18 - Deer polypeptide sequence encoded by DACC-9.
SEQ ID NO:19 - Human polypeptide orthologous to SEQ ID NO:18 (Accession No. XP_059039).
SEQ ID NO:20 - Rat polypeptide orthologous to SEQ ID NO:18 (Accession No. P97541).
20 SEQ ID NO:21 - Deer polypeptide sequence encoded by DACC-10.
SEQ ID NO:22 - Human polypeptide orthologous to SEQ ID NO:21 (Accession No. NP_000384).
SEQ ID NO:23 - Mouse polypeptide orthologous to SEQ ID NO:21 (Accession No. NP_031763).
25 SEQ ID NO:24 - Deer polypeptide sequence encoded by DACC-11.
SEQ ID NO:25 - Human polypeptide orthologous to SEQ ID NO:24 (Accession No. AAB94054).
SEQ ID NO:26 - Mouse polypeptide orthologous to SEQ ID NO:24 (Accession No. P11087).
30 SEQ ID NO:27 - Human transthyretin (Accession No. P02766).
SEQ ID NO:28 - Deer cDNA sequence of clone DACC-2.
SEQ ID NO:29 - Deer cDNA sequence of clone DACC-3.
SEQ ID NO:30 - Deer cDNA sequence of clone DACC-4.
SEQ ID NO:31 - Deer cDNA sequence of clone DACC-5.
35 SEQ ID NO:32 - Deer cDNA sequence of clone DACC-6.
SEQ ID NO:33 - Deer cDNA sequence of clone DACC-7.

SEQ ID NO:34 - Deer cDNA sequence of clone DACC-8.

SEQ ID NO:35 - Deer cDNA sequence of 5' end of clone DACC-9.

SEQ ID NO:36 - Deer cDNA sequence of 3' end of clone DACC-9.

SEQ ID NO:37 - Deer cDNA sequence of clone DACC-10.

5 SEQ ID NO:38 - Deer cDNA sequence of clone DACC-11.

SEQ ID NO:39 - Oligonucleotide primer.

SEQ ID NO:40 - Oligonucleotide primer.

SEQ ID NO:41 - N-terminal sequence of deer transthyretin protein fragment.

SEQ ID NO:42 - N-terminal sequence of deer transthyretin protein fragment.

10

Detailed Description of the Invention:

General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilised in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

25

Polypeptides

By "substantially purified" we mean a polypeptide that has been separated from the lipids, nucleic acids, other polypeptides, and other contaminating molecules with which it is associated in its native state.

30 The % identity of a polypeptide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 15 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 15 amino acids. More preferably, the query sequence is at least 50 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 50 amino acids. Even more preferably, the query sequence is at

35

least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. More preferably, the query sequence is at least 250 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 250 amino acids. Even more preferably, the query sequence is at least 500 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 500 amino acids.

As used herein a "biologically active fragment" of a polypeptide used in the methods of the present invention is a portion of the polypeptide which maintains the ability to stimulate animal cell growth and/or division.

Polypeptides useful for the methods of the present invention can either be naturally occurring or mutants and/or fragments thereof.

Amino acid sequence mutants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such mutants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics.

In designing amino acid sequence mutants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting other residues adjacent to the located site.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Substitution mutants have at least one amino acid residue in the polypeptide molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active and/or binding site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically

conserved sites, are preferably substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "exemplary substitutions".

5

TABLE 1

Original Residue	Exemplary Substitutions
Ala (A)	val; leu; ile
Arg (R)	lys
Asn (N)	gln; his;
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn; his
Glu (E)	asp
Gly (G)	pro
His (H)	asn; gln
Ile (I)	leu; val; ala; norleucine
Leu (L)	norleucine, ile; val; met; ala; phe
Lys (K)	arg
Met (M)	leu; phe;
Phe (F)	leu; val; ala
Pro (P)	gly
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe
Val (V)	ile; leu; met; phe ala; norleucine

Furthermore, if desired, unnatural amino acids or chemical amino acid analogues can be introduced as a substitution or addition into the polypeptide. Such amino acids include, but are not limited to, the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-aminobutyric acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogues in general.

Also included within the scope of the invention are polypeptides which are differentially modified during or after synthesis, e.g., by biotinylation, benzylation, glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. These modifications may serve to increase the stability and/or bioactivity of the polypeptide.

Polypeptides can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated polypeptide of the present invention is produced by culturing a cell capable of expressing the polypeptide under conditions effective to produce the polypeptide, and recovering the polypeptide. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a polypeptide of the present invention. Such medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art.

Polynucleotides

By "isolated polynucleotide" we mean a polynucleotide separated from the polynucleotide sequences with which it is associated or linked in its native state. Furthermore, the term "polynucleotide" is used interchangeably herein with the term "nucleic acid molecule".

The % identity of a polynucleotide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 45 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 45 nucleotides. Preferably, the query sequence is at least 150 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 150 nucleotides. More preferably, the query sequence is at least 300 nucleotides in length and the GAP analysis aligns the two sequences over a region of at least 300 nucleotides.

As used herein, high stringency conditions are those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% NaDodSO₄ at 50°C; (2) employ during hybridisation a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS and 10% dextran sulfate at 42°C in 0.2 x SSC and 0.1% SDS.

Polynucleotides may possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the nucleic acid). It is thus apparent that polynucleotides can be either naturally occurring or recombinant.

Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for the formation of a stable hybrid between an oligonucleotide and a complementary sequence on a nucleic acid molecule of the present

invention. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules or used to regulate the production of polypeptides as disclosed herein (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). Oligonucleotide used as a probe are typically conjugated with a label such as a radioisotope, an enzyme, biotin, a fluorescent molecule or a chemiluminescent molecule.

Catalytic Nucleic Acids

10 The term catalytic nucleic acid refers to a DNA molecule or DNA-containing molecule (also known in the art as a "deoxyribozyme") or an RNA or RNA-containing molecule (also known as a "ribozyme") which specifically recognizes a distinct substrate and catalyzes the chemical modification of this substrate. The nucleic acid bases in the catalytic nucleic acid can be bases A, C, G, T and U, as well as derivatives thereof. Derivatives of these bases are well known in the art.

Typically, the catalytic nucleic acid contains an antisense sequence for specific recognition of a target nucleic acid, and a nucleic acid cleaving enzymatic activity (also referred to herein as the "catalytic domain"). The types of ribozymes that are particularly useful in this invention are the hammerhead ribozyme (Haseloff and Gerlach 1988, Perriman et al., 1992) and the hairpin ribozyme (Shippy et al., 1999).

The ribozymes of this invention and DNA encoding the ribozymes can be chemically synthesized using methods well known in the art. The ribozymes can also be prepared from a DNA molecule (that upon transcription, yields an RNA molecule) operably linked to an RNA polymerase promoter, e.g., the promoter for T7 RNA polymerase or SP6 RNA polymerase. Accordingly, also provided by this invention is a nucleic acid molecule, i.e., DNA or cDNA, coding for the ribozymes of this invention.

30 When the vector also contains an RNA polymerase promoter operably linked to the DNA molecule, the ribozyme can be produced *in vitro* upon incubation with RNA polymerase and nucleotides. In a separate embodiment, the DNA can be inserted into an expression cassette or transcription cassette. After synthesis, the RNA molecule can be modified by ligation to a DNA molecule having the ability to stabilize the ribozyme and make it resistant to RNase.

35 Alternatively, the ribozyme can be modified to the phosphothio analog for

use in liposome delivery systems. This modification also renders the ribozyme resistant to endonuclease activity.

5

dsRNA

dsRNA is particularly useful for specifically inhibiting the production of a particular protein. Although not wishing to be limited by theory, Dougherty and Parks (1995) have provided a model for the mechanism by which dsRNA can be used to reduce protein production. This model has recently been modified and expanded by Waterhouse et al. (1998). This technology relies on the presence of dsRNA molecules that contain a sequence that is essentially identical to the mRNA of the gene of interest, in this case an mRNA encoding a polypeptide useful in the methods of the present invention. Conveniently, the dsRNA can be produced in a single open reading frame in a recombinant vector or host cell, where the sense and anti-sense sequences are flanked by an unrelated sequence which enables the sense and anti-sense sequences to hybridize to form the dsRNA molecule with the unrelated sequence forming a loop structure. The design and production of suitable dsRNA molecules for the present invention is well within the capacity of a person skilled in the art, particularly considering Dougherty and Parks (1995), Waterhouse et al. (1998), WO 99/32619, WO 99/53050, WO 99/49029, and WO 01/34815.

25 Recombinant Vectors

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule encoding a polypeptide useful for the methods of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules encoding a polypeptide useful for the methods of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid.

One type of recombinant vector comprises a nucleic acid molecule encoding a polypeptide useful for the methods of the present invention operably linked to an expression vector. The phrase operably linked refers to insertion of a nucleic acid molecule into an expression vector in a manner
5 such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic
10 or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in recombinant cells of the present invention, including in bacterial, fungal, endoparasite, arthropod, other animal, and plant cells. Preferred expression vectors useful for the methods of the present invention
15 can direct gene expression in bacterial; yeast, arthropod and mammalian cells and more preferably in the cell types disclosed herein. Most preferably, vectors useful for the methods of the present invention can direct gene expression in mammalian cells.

Expression vectors of the present invention contain regulatory
20 sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules useful for the methods of the present invention. In particular, recombinant molecules of the present invention include
25 transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences
30 include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, arthropod and mammalian cells, such as, but not limited to,
35 tac, lac, trp, trc, oxy-pro, omp/lpp, rrnB, bacteriophage lambda, bacteriophage T7, T7lac, bacteriophage T3, bacteriophage SP6, bacteriophage SP01,

- metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus,
- 5 cytomegalovirus (such as intermediate early promoters), simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-
- 10 specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention are most preferably naturally occurring transcription control sequences naturally associated with mammals.
- 15 Recombinant molecules of the present invention may also (a) contain secretory signals (i.e., signal segment nucleic acid sequences) to enable an expressed polypeptide useful for the methods of the present invention to be secreted from the cell that produces the polypeptide and/or (b) contain fusion sequences which lead to the expression of fusion proteins. Examples of
- 20 suitable signal segments include any signal segment capable of directing the secretion of the fusion protein. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments, as well as natural signal sequences. Suitable fusion segments encoded by
- 25 fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule useful for the methods of the present invention can be joined to a fusion segment that directs the encoded protein to the proteasome, such as a ubiquitin fusion segment. Recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the
- 30 nucleic acid sequences.

Host cells

- Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant
- 35 molecules useful for the methods of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by

which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained.

Suitable host cells to transform include any cell that can be transformed with a polynucleotide of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention). Host cells useful for the methods of the present invention either can be endogenously (i.e., naturally) capable of producing the expressed protein or can be capable of producing such proteins after being transformed with an expression vector as disclosed herein. Host cells of the present invention can be any cell capable of producing at least one protein useful for the methods of the present invention, and include bacterial, fungal (including yeast), parasite, arthropod, plant and animal cells. Most preferably, the host cell is a mammalian cell.

Suitable prokaryotes include, but are not limited to, eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Escherichia coli*, *Bacilli* such as *B. subtilis* or *B. thuringiensis*, *Pseudomonas* species such as *P. aeruginosa*, *Salmonella typhimurium* or *Serratia marcescens*.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for expressing the protein(s) of the present invention. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as e.g. *K. lactis*; filamentous fungi such as, e.g. *Neurospora*, or *Penicillium*; and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

Suitable higher eukaryotic host cells can be cultured vertebrate, invertebrate or plant cells. Insect host cells from species such as *Spodoptera frugiperda*, *Aedes aegypti*, *Aedes albopictus*, *Drosophila melanogaster*; and *Bombyx mori* can be used. Plant cell cultures of cotton, corn, potato, soybean,

tomato, and tobacco can be utilised as hosts. Typically, plant cells are transfected by incubation with certain strains for the bacterium *Agrobacterium tumefaciens*.

Propagation of animal cells in culture (tissue culture) has become a routine procedure in recent years. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture); baby hamster kidney cells (BHK ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO); mouse sertoli cells, monkey kidney cells (CV1 ATCC CCL 70); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK ATCC CCL 34), and a human hepatoma cell line (Hep G2). Preferred host cells are human embryonic kidney 293 and Chinese hamster ovary cells.

The host cell may also be selected from mammalian foetal cells, particularly human foetal cells. Especially preferred are chondrocytes including human chondrocytes, or other mesenchymal cells including human mesenchymal stem cells. Such transformed or transfected host cells may be used for, for example, xenotransplantation (i.e. where the host cell is of other mammalian origin) or autotransplantation (i.e. where the host cell originates from the recipient) to a human subject.

Host cells are transfected and preferably transformed with expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells.

Recombinant DNA technologies can be used to improve the expression of transformed polynucleotide molecules by manipulating, for example, the number of copies of the polynucleotide molecules within a host cell, the efficiency with which those polynucleotide molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of polynucleotide molecules useful for the methods of the present invention include, but are not limited to, operably

linking polynucleotide molecules to high-copy number plasmids, integration of the polynucleotide molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of polynucleotide molecules of the present invention to correspond to the codon usage of the host cell, and the deletion of sequences that destabilize transcripts. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing polynucleotide molecules encoding such a protein.

Gene Therapy

The polynucleotides, polypeptides, agonists and antagonists that are polypeptides, may be employed in accordance with the present invention by expression of such polypeptides in treatment modalities often referred to as "gene therapy". Thus, for example, cells from a patient may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*. The engineered cells can then be provided to a patient to be treated with the polypeptide. In this embodiment, cells may be engineered *ex vivo*, for example, by the use of a retroviral plasmid vector containing RNA encoding a polypeptide useful for the methods of the present invention can be used to transform stem cells or differentiated stem cells. Such methods are well-known in the art and their use in the present invention will be apparent from the teachings herein.

Further, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by procedures known in the art. For example, a polynucleotide useful for a method of the present invention may be engineered for expression in a replication defective retroviral vector or adenoviral vector or other vector (e.g., poxvirus vectors). The expression construct may then be isolated. A packaging cell is transduced with a plasmid vector containing RNA encoding a polypeptide useful for a method of the present invention, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention

should be apparent to those skilled in the art from the teachings of the present invention.

Retroviruses from which the retroviral plasmid vectors hereinabove-mentioned may be derived include, but are not limited to, Moloney Murine
5 Leukemia Virus, Spleen Necrosis Virus, Rous Sarcoma Virus, Harvey
Sarcoma Virus, Avian Leukosis Virus, Gibbon Ape Leukemia Virus, Human
Immunodeficiency Virus, Adenovirus, Myeloproliferative Sarcoma Virus, and
Mammary Tumor Virus. In a preferred embodiment, the retroviral plasmid
vector is derived from Moloney Murine Leukemia Virus.

10 Such vectors will include one or more promoters for expressing the
polypeptide. Suitable promoters which may be employed include, but are
not limited to, the retroviral LTR; the SV40 promoter; and the human
cytomegalovirus (CMV) promoter. Cellular promoters such as eukaryotic
cellular promoters including, but not limited to, the histone, RNA polymerase
15 III, and β -actin promoters, can also be used. Additional viral promoters
which may be employed include, but are not limited to, adenovirus
promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters.
The selection of a suitable promoter will be apparent to those skilled in the
art from the teachings contained herein.

20 The nucleic acid sequence encoding the polypeptide useful for a
method of the present invention will be placed under the control of a suitable
promoter. Suitable promoters which may be employed include, but are not
limited to, adenoviral promoters, such as the adenoviral major late promoter;
or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the
25 respiratory syncytial virus (RSV) promoter; inducible promoters, such as the
MMT promoter, the metallothionein promoter; heat shock promoters; the
albumin promoter; the ApoAI promoter; human globin promoters; viral
thymidine kinase promoters, such as the Herpes Simplex thymidine kinase
promoter; retroviral LTRs (including the modified retroviral LTRs herein
30 above described); the β -actin promoter; and human growth hormone
promoters. The promoter may also be the native promoter which controls the
gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell
lines to form producer cell lines. Examples of packaging cells which may be
35 transfected include, but are not limited to, the PE501, PA317, Y-2, Y-AM,
PA12, T19-14X, VT-19-17-H2, YCRE, YCRIP, GP+E-86, GP+envAm12, and

DAN cell lines as described in Miller (1990). The vector may be transduced into the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line will generate infectious retroviral vector particles, which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles may then be employed to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, mesenchymal cells, chondrocytes, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

Genetic therapies in accordance with the present invention may involve a transient (temporary) presence of the gene therapy polynucleotide in the patient or the permanent introduction of a polynucleotide into the patient.

Genetic therapies, like the direct administration of agents discussed above, in accordance with the present invention may be used alone or in conjunction with other therapeutic modalities.

25 Compositions and Administration

Compositions useful for a method of the present invention comprise an acceptable carrier. Typically, the carrier will also be considered as a "pharmaceutically acceptable carrier", meaning that it is suitable to be administered to an mammal, preferably a human. Suitable carriers include isotonic saline solutions, for example phosphate-buffered saline.

The composition of the invention may be administered by direct injection. The composition may be formulated for, as examples, parenteral, intramuscular, intravenous, subcutaneous, intraocular, oral or transdermal administration. Typically, each protein (for example) may be administered at a dose of from 0.01 to 30 mg/kg body weight, preferably from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight. The routes of

administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular, compound, animal and condition.

5 Polynucleotides/vectors encoding polypeptide components for use in affecting viral infections may be administered directly as a naked nucleic acid construct, preferably further comprising flanking sequences homologous to the host cell genome. When the polynucleotides/vectors are administered as a naked nucleic acid, the amount of nucleic acid administered may typically
10 be in the range of from 1 μ g to 10 mg, preferably from 100 μ g to 1 mg. Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example
15 lipofectamTM and transfectamTM). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition useful for a method of the present invention into an animal. Suitable controlled release
20 vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

25

Methods of Screening for Modulators of Polypeptide Activity

As used herein a "lead compound" is an agent identified by the methods of the present invention which is subject to trials with the goal of ultimately being formulated in, for example, a composition and sold as an
30 agent for stimulating cell growth and/or division.

Known screening techniques can be used to identify agents which modulate the activity, or production of, a polypeptide of the present invention which stimulates cell growth and/or division. For instance, a candidate agents can be exposed to a cell in the presence or absence of the
35 polypeptide, and the resulting effects on cell growth and/or division analysed, through standard techniques such as measuring cell numbers or DNA

synthesis, to determine if the candidate agent directly effects the activity of the polypeptide,

Another method for screening for agonists/antagonists involves mixing the polypeptide with a binding partner (which is capable of binding to the polypeptide) and measuring their binding to each other in the presence or absence of a potential agonist/antagonist. The polypeptide or the binding partner can be detectably labeled using known labels such as those selected from the group consisting of: radioisotopes, fluorophores and chromophores. This binding assay may be in the form of an ELISA plate assay. There are other binding formats known to those of skill in the art, including coprecipitation, centrifugation and surface plasmon resonance.

One potential antagonist is a small molecule which binds to the polypeptide. Examples of small molecules include, but are not limited to, small peptides, peptide-like molecules, plant secondary metabolites or synthetic organic chemicals.

As described herein, suitable antisense polynucleotide and dsRNA molecules can be designed based on the sequences of a polynucleotide encoding the polypeptide. Such antisense polynucleotide and dsRNA molecules can be used as agents for modulating cell growth and/or division when a cell has transformed with the antisense polynucleotide or dsRNA molecule.

Such antisense polynucleotides and dsRNA molecules can also be screened for use as an agent using the methods of the present invention. For instance, a polynucleotide encoding the polypeptide of interest can be expressed in a cell system, or a cell-free expression system, resulting in the production of the polypeptide. Candidate antisense polynucleotides and dsRNA molecules designed based on the can be incorporated into the system and the resulting affects on transcribed mRNA levels or polypeptide levels or activity, can readily be measured using techniques known in the art.

Suitable inhibitors of a polypeptide's ability to stimulate cell growth and/or division are compounds that interact directly with a protein's active site, thereby inhibiting activity.

Effective amounts and dosing regimens for the application of agents identified by the methods of the present invention can readily be determined using techniques known to those skilled in the art.

Phage Libraries for Candidate Agent Screening

Phage libraries can be constructed which when infected into host *E. coli* produce random peptide sequences of approximately 10 to 15 amino acids. Specifically, the phage library can be mixed in low dilutions with
5 permissive *E. coli* in low melting point LB agar which is then poured on top of LB agar plates. After incubating the plates at 37°C for a period of time, small clear plaques in a lawn of *E. coli* will form which represents active phage growth and lysis of the *E. coli*. A representative of these phages can be absorbed to nylon filters by placing dry filters onto the agar plates. The filters
10 can be marked for orientation, removed, and placed in washing solutions to block any remaining absorbent sites. The filters can then be placed in a solution containing, for example, a radioactively labeled polypeptide useful for the methods of the present invention (e.g., a polypeptide having an amino acid sequence comprising SEQ ID NO:1). After a specified incubation period,
15 the filters can be thoroughly washed and developed for autoradiography. This allows plaques containing the phage that bind to the radioactive polypeptide to be detected. These phages can be further cloned and then retested for their ability to bind to the polypeptide as before. Once the phages have been purified, the binding sequence contained within the phage can be
20 determined by standard DNA sequencing techniques. Once the DNA sequence is known, synthetic peptides can be generated which represents these sequences.

The effective peptide(s) can be synthesized in large quantities for use in *in vivo* models and eventually as an agent for modulating cell growth and/or
25 division. It should be emphasized that synthetic peptide production is relatively non-labor intensive, easily manufactured, quality controlled and thus, large quantities of the desired product can be produced rather cheaply.

Hybrid Screening Techniques

30 In yet another embodiment of the invention, the polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, for example, U.S. 5,283,317 and WO94/10300), to identify other proteins, which bind to or interact with the polypeptide and are involved in modulating cell growth and/or division.

35 The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation

domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for the polypeptide of interest is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the polypeptide of interest.

Protein-Structure Based Design of Candidate Agents

Crystals of a polypeptide useful for the methods of the present invention could be grown by a number of techniques including batch crystallation, vapour diffusion (either by sitting drop or hanging drop) and by microdialysis. Seeding of the crystals in some instances could be required to obtain X-ray quality crystals. Standard micro and/or macro seeding of crystals may therefore be used. Once a crystal is grown, X-ray diffraction data can be collected using standard techniques.

Once the three-dimensional structure of the polypeptide is determined, a potential antagonist or agonist can be examined through the use of computer modeling using a docking program such as GRAM, DOCK, or AUTODOCK (Dunbrack et al., 1997). This procedure can include computer fitting of potential ligands to the polypeptide to ascertain how well the shape and the chemical structure of the potential ligand will complement or interfere with the activity of the polypeptide. Computer programs can also be employed to estimate the attraction, repulsion, and steric hindrance of the ligand to the polypeptide. Generally the tighter the fit (e.g., the lower the steric hindrance, and/or the greater the attractive force) the more potent the potential agent will be since these properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential agent the more likely that the agent will not interfere with other

proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Initially a potential compound could be obtained, for example, by screening a random peptide library produced by a recombinant bacteriophage as described above, or a chemical library. A compound selected in this manner could be then be systematically modified by computer modeling programs until one or more promising potential compounds are identified.

Such computer modeling allows the selection of a finite number of rational chemical modifications, as opposed to the countless number of essentially random chemical modifications that could be made, and of which any one might lead to a useful agent. Each chemical modification requires additional chemical steps, which while being reasonable for the synthesis of a finite number of compounds, quickly becomes overwhelming if all possible modifications needed to be synthesized. Thus through the use of the three-dimensional structure and computer modeling, a large number of these compounds can be rapidly screened on the computer monitor screen, and a few likely candidates can be determined without the laborious synthesis of untold numbers of compounds.

The prospective agent can be placed into any standard binding assay to test its effect.

METHODS

General

Radionucleotides

Alpha linked radioactive phosphorus [$\alpha^{32}\text{P}$] 2'-deoxycytidine 5'-triphosphate (dCTP), gamma linked [$\gamma^{32}\text{P}$] 2'-deoxyadenosine 5'-triphosphate (dATP), [$\alpha^{32}\text{P}$] 2'-deoxyuridine (dUTP) and [$\alpha^{35}\text{S}$] dATP nucleotides were obtained from Dupont NEN[®] (Wilmington, DE, USA).

Restriction enzymes

All restriction enzymes used were obtained from Roche (Roche Molecular Systems, Inc., NJ, USA).

Primers

All primers were commercially obtained from Bresatec Limited (SA, Australia) and were received in desiccated form. Pellets were resuspended in

sterile water (Baxter, NSW) to a concentration of 1 mg/mL and stored at -70°C. Working solutions at 100 ng/mL were diluted from this stock concentration and stored at 4°C.

5

Polymerase Chain Reaction (PCR)

The reagents used for PCR were obtained from three main sources. For most PCR reactions, MgCl₂ solution of 25 mM, 10 x Taq polymerase were
10 obtained from Perkin-Elmer (Roche Molecular Systems, Inc., NJ, USA). Where greater sensitivity of PCR was needed an Advantage® cDNA PCR kit (CLONTECH Laboratories, Inc., USA) or a PLATINUM® Taq DNA polymerase High Fidelity (Gibco BRL, Life Technologies) was used. A Perkin Elmer Cetus DNA thermal cycler machine was used and the number of cycles applied was
15 dependent on the type of polymerase used and the nature of the reaction. The most common cycles used were 94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 55°C for 39 s, 72°C for 1 min.

Agarose Gel Electrophoresis

20 All agarose gels were made using 1 x TAE (40 mM Tris-acetate, 1 mM EDTA pH 8.0) for both the gel and running buffer. 1% agarose/TAE gels were made using agarose type I (Sigma Chemical Co., St. Louis, MO, USA). Loading buffer for all samples consisted of 0.25% bromophenol blue and 40% (w/v) sucrose in water. A concentration of 0.5 µg/mL of ethidium bromide
25 was used for each gel.

Two types of horizontal gel apparatus were used. For 30 ml gels, a HORIZON® 58 Gel tank (BRL, Life Technologies Inc, Gaithersburg, MD, USA) was used. An Extra-wide Minigel system model D2 (Owl Scientific Plastics Inc, Cambridge, MA, USA) was used for 70 mL gels in analysing larger
30 numbers of samples. Samples were electrophoresed using a LKB, Bromma power pack 2197 (Uppsala, Sweden) or an EPS 600 power pack (Pharmacia Biotech, Sweden) at 80 to 120 V and a time range of 20 to 60 min.

Purification and concentration of DNA

35 To purify and concentrate DNA after restriction digestion or from agarose gels, a QIAquick® Gel extraction Kit (QIAGEN Pty Ltd, Vic, Australia).

DNA bands of interest on agarose gels were isolated in 1.5 mL microcentrifuge tubes. The gel slice was then incubated at 50°C until complete melting of the agarose and processed as per kit instructions. For DNA to be purified from restriction enzymes, no incubation at 50°C was
5 required, instead was processed directly as per kit instructions. The purified DNA samples were stored at 4°C.

Preparation of plasmid DNA

Minipreparation of plasmid DNA

10 For small amounts of plasmid DNA, a Wizard® Plus Minipreps DNA Purification System (Promega Corp., NSW, Australia) was used. This system came as a kit, providing a reliable method for good quality plasmid DNA. Three microlitres of bacterial culture in LB with the appropriate supplements was inoculated from a colony or pure culture and incubated at 37°C overnight
15 with shaking. One point five microlitres of overnight bacterial culture was placed in a 1.5 mL microfuge tube and spun in a microcentrifuge for 30 s, after which the supernatant was discarded. The cell pellet was then processed as per kit instructions. The DNA was eluted in 50 µL of 1 x TE (1 M Tris/0.5 M EDTA, pH 8.0). The quality of the DNA was analysed by test
20 digestion of 5 µL with appropriate restriction enzymes and running on an agarose gel.

Midipreparation of plasmid DNA

For larger amounts of plasmid DNA and to prepare DNA for
25 sequencing, a QIAGEN® Plasmid Midi kit was used. 25 mL of LB with appropriate supplements was inoculated with a pure bacterial colony and incubated with shaking at 37°C overnight. The overnight culture was transferred into a 250 mL centrifuge bottle and spun in a Beckmann® XL-90 (Beckmann Instruments, Inc., CA, USA) or Sorvall® RC 5C Plus (Dupont
30 Australia Ltd., Sydney, Australia) ultracentrifuge at 8,000 rpm for 10 min to pellet the bacteria. The bacterial pellet was then processed as per kit instructions. The DNA pellet was redissolved in 200 µL of 1 x TE. Spectrophotometer readings (on a Beckmann Du®-68 machine) were taken to determine the yield of plasmid DNA. DNA concentration was calculated
35 using the formula: 1.0 unit of optical density at 260 nm is equivalent to 50 µg/mL dsDNA.

DNA Sequencing

High purity double stranded DNA template for sequencing was generated by the above procedure. This template was sent to SUPAMAC
5 (Sydney University and Prince Alfred Macromolecular Analysis Centre, Sydney, Australia) or AGRF (Australian Genome Research Facility, Brisbane, Australia) where the template was sequenced by dye-terminator chemistry. With this method, 4 dye-labelled dideoxy nucleotides replace standard dideoxy nucleotides, incorporating into the DNA as the terminating base.
10 Universal primers T7, SP6, T3, and -21M13 (Forward and Reverse) were used in the cycle sequencing reaction. The fluorescent signal for each base was tracked to produce an electropherogram file, displaying different bases of the sequence as peaks, where individual peaks were labelled with one of four different colours corresponding to the four bases (A, G, C, and T). This file of
15 raw data was obtained for analysis. The sequence data was analysed using the Sequencher® program (version 3, Genes Codes Corp., Ann Arbor, MI, USA).

RNA Techniques

20 All reagents were made using diethylpyrocarbonate (DEPC) treated water. Dedicated glassware and pipette tips were used, and gloves were worn at all times to minimise the risk of contamination by RNases.

RNA probes

25 To create an RNA probe, it was necessary to clone the cDNA product into a suitable vector (such as pGEM T-Easy®) that contained RNA polymerase binding sites (such as SP6 and T7), allowing single stranded RNA to be manufactured. This was transformed into host bacteria (as described later) and plasmid was obtained by miniprep (as described previously).

30

Radioactively labelled cRNA probe preparation

Two micrograms of linearised DNA template containing the insert cloned in a suitable vector was combined in a screw-topped tube with 4 µL of
5 x transcription buffer (200 mM Tris HCl, pH7.5; 30 mM MgCl₂; 25 mM
35 NaCl), 2 µL of 0.2 M dithiothreitol (DTT), 1 µL of rRNAsin® RNase inhibitor (Promega Corp., Madison, WI, USA), 4 µL of ATP, CTP, GTP (Pharmacia LKB

Biotechnology, Boronia, Australia) mixture was added. This mixture was vortexed and spun down. Then, 2.5 μL of $\alpha^{32}\text{P}$ -dUTP and 2.5 μL of appropriate RNA polymerase was added to the mixture and incubated at 37°C for 1 h. The DNA template was degraded with 5 μL of mixture containing 200
5 units of DNaseI (GibcoBRL Life Technologies), 9 μL of DEPC water and 2.5 units of rRNasin at 37°C for 10 min.

The radiolabelled riboprobe was purified using an Elutip-D column as per manufacturer's instructions (Schleicher and Schell, Dassel, Germany). The radioactive product was eluted in 300 μL of high salt buffer (1 M NaCl;
10 0.01 M Tris, pH 8.0; 1 mM EDTA, pH 8.0). Two microlitres were removed from the 300 μL and used to measure the radioactivity of the probe on a \square counter (Tricarb™ Liquid Scintillation Analyser 1600TR, Packard Instruments Co., Canberra, Australia). Only those with a measured radioactivity of at least 50,000 cpm/ μL were used for hybridisation. The rest
15 was immediately frozen at -70°C and was used within 24 h.

RNA Preparation

Cell and tissue specimen preparation

Human foetal cartilage tissue was provided by Dr. Bernie Tuch (Prince
20 of Wales Hospital, Sydney, Australia) and Dr. Sue Craig (Royal North Shore Hospital, Sydney, Australia). Their collection and use for this study was approved by the Royal North Shore Hospital Human Research Ethics Committee (HREC). Cartilage samples of adult deer antler (whole and regions), adult deer articular cartilage, 6 week old sheep growth plate
25 cartilage, 6 week old sheep articular cartilage, 6 week old sheep sternal cartilage, adult human articular cartilage, 4 weeks to term foetal male deer epiphyseal cartilage, 4 weeks to term foetal male deer intervertebrate disc cartilage, 4 weeks to term foetal male deer rib cartilage, 4 weeks to term foetal male deer sternal cartilage, and 4 weeks to term foetal male deer calvaria
30 cartilage (were provided by Mr Denis White of ADP Pharmaceutical Pty Limited, Goulburn, NSW, Australia) were taken for RNA analysis. These samples were either collected by snap freezing in liquid nitrogen (as with the whole deer antler, skin removed) or were enzymatically digested first to release cells, the chondrocytes collected by centrifugation then snap frozen in
35 liquid nitrogen. The enzymatic digestion procedure was the preferred

method for preparation of RNA as extraction directly from snap frozen tissue gave very low RNA yields.

A typical procedure was performed as follows: Immediately after sacrifice (or in the case of deer antler, after harvesting from the animal after administering local anaesthetic (Lignocaine) to front and back veins) specimens were transported to the laboratory in plastic bags maintained at 4°C on ice. The specimens were thoroughly sprayed with 70% (v/v) ethanol and surrounding tissue (in particular, mesenchymal) was carefully removed under sterile conditions to obtain only target cartilage. The deer antler cartilage (DAC) regions were discernible by the pre-chondrocyte tissue observed as white, soft cartilage with no blood vessels; the mature chondrocyte tissue observed as soft cartilage with blood vessels; and the hypertrophic chondrocyte tissue observed as hard mineralised cartilage full of blood vessels. The outer rim of cartilage (intramembranous ossification) was discarded in each DAC region.

The cartilage was digested for 2h at 37°C in 0.1% (w/v) pronase (Boehringer Mannheim Australia Pty Ltd, Castle Hill, NSW, Australia) in Hams F12 media (Trace Biosciences Pty Ltd, Castle Hill, NSW, Australia) supplemented with 10% (v/v) foetal bovine serum (Trace Biosciences), 76 mM NaHCO₃, 20 mM HEPES (Sigma Chemical Company, St Louis, MI, USA) and 80 units per mL gentamycin (Delta West Pty Ltd, WA, Australia). This was then replaced with media containing 0.04 % (w/v) collagenase (Sigma) for digestion overnight at 37°C. For DAC, the digestion procedure was 0.125% (w/v) trypsin (Sigma) in 1:1 DMEM (Sigma)/Hams F12 (DMEM:F12) media supplemented with 76 mM NaHCO₃, 20 mM HEPES, 80 units per mL gentamycin at 4 °C overnight, then 37°C for 1 h. This was replaced with media containing 0.04% (w/v) collagenase and supplemented with 10% (v/v) foetal bovine serum at 37°C for 3-4 h, vortexing for 10 sec every 30 min. Cells were collected through a sterile 70 µm Cell Strainer (Becton Dickinson, Franklin Lakes, NJ, USA) and pelleted for RNA extraction.

Extraction of Total RNA

Cell pellets (or in the case of whole deer antler, tissue samples) were removed from the -70°C freezer and placed on dry ice. The tissue sample of whole deer antler was homogenised first in mortars filled with liquid nitrogen. The cell pellet (10 x 10⁶ cells) or 50 mg tissue sample was sonicated

after adding 1 mL of TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH, USA). TRI Reagent® was used as it has a higher recovery of undegraded mRNAs than other RNA extraction methods, which was essential for this analysis. The total RNA was then extracted from samples using the
5 manufacture's protocol (TRI Reagent - RNA, DNA, and protein isolation reagent. Manufacturer's protocol (1995), Molecular Research Center). The final dried total RNA pellet was resuspended into 50 µL of DEPC treated water and stored at -70°C.

10 *Quantification of RNA*

Spectrophotometer readings (on a Beckmann Du®-68 machine) were taken to determine the yield of RNA. RNA concentration was calculated using the formula: 1.0 unit of optical density at 260 nm is equivalent to 40 µg/mL RNA.

15

Northern Blot Analysis

Northern Blot Preparation

Total RNA samples (5 µg) were vacuum dried and resuspended into 15 µL of blue juice mix loading buffer, consisting of 20% (v/v) formaldehyde,
20 40% (v/v) deionised formamide, 1 x MOPS (200 mM MOPS (Sigma), 50 mM Na acetate, 10 mM disodium EDTA, pH 7.0) and 12% (v/v) "blue juice" (50% (v/v) glycerol (Ajax Chemicals, Auburn, NSW, Australia), 1% (v/v) EDTA, 0.4% (v/v) bromophenol blue (International Biotechnologies Inc., New Haven, Connecticut, USA)). The samples were denatured at 65°C for 3 minutes then
25 were fractionated by electrophoresis at 110 V for 4-4.5 h on a 1% (w/v) agarose (2.2 M formaldehyde) gel. 0.24 - 9.5 kb RNA ladder (GibcoBRL Life Technologies, Gaithersburg, Maryland, USA) was also included. The gel was stained with ethidium bromide and transilluminated with ultraviolet light to visualise the 28S and 18 S rRNA. After photographing, the gel was rinsed in
30 20 x SSC (3 M NaCl and 0.3 M Na citrate) for 10 min. The gel was then turned upside down onto a 3 MM Whatman paper which was used as a wick. Any air bubbles were rolled out and a Genescreen® nylon membrane (DuPont, NEN, Boston, MA, USA) of the same dimension was placed on the top of the gel to transfer the total RNA from the gel to the membrane overnight. The
35 nylon membrane was then carefully removed and exposed to UV light to crosslink the RNA to the membrane by using an energy level of 120 mJ in a

UV Stratalinker® 1800 (Stratagene Corp., La Jolla, California, USA). The membrane was sealed in a plastic bag while the membrane was still moist.

Northern Blot Hybridisation

5 The blot was hybridised using the Hybaid® hybridisation bottle system with the Hybaid® hybridisation oven (Hybaid, Middlesex, United Kingdom) as this system gave sensitive and reproducible results. Before hybridising with the radiolabelled probe, the blot was soaked with 2 x SSC (0.3 M NaCl and 0.03 M Na citrate) then prehybridised with 10 mL prehybridisation buffer
10 containing 50% (v/v) deionised formamide; 0.8 M NaCl; 1 mM EDTA, pH 7.4; 50 mM PO₄, pH 6.5; 2% (w/v) SDS; 2.5 x Denhardts solution (100 x Denhardts solution consisting of 2% (v/v) Ficoll (Sigma), 2% (w/v) polyvinylpyrrolidone (Sigma) and 2% (w/v) bovine serum albumin); 100 mg/mL sheared salmon sperm DNA (Sigma); 200 mg/mL tRNA (last two
15 reagents were denatured by heating to 95°C for 5 min prior to addition) at 65°C for 3 h with continuous rotation. This temperature (65°C) was used for prehybridisation, hybridisation and washing to ensure high stringency conditions for annealing of probe to target RNA.

 The radiolabelled cRNA probe (5 x 10⁶ counts/mL) was thawed quickly
20 at room temperature and injected directly into the hybridisation bottle containing the prehybridisation buffer. Hybridisation was carried out with continuous rotation at 65°C overnight. Following hybridisation, the blot was washed twice in 100 mL of a buffer containing 0.1 x SSC and 1% (w/v) SDS at 65°C continuous rotation for 15 min. After washing, the moist blot was
25 sealed in a plastic bag and exposed to a phosphorimager screen for between 24 h and 7 days. Scanning of the image was performed using the ImageQuant software program (Molecular Dynamics, USA).

cRNA Probes

30 Collagen Type II (HC22)

 The cDNA was kindly supplied by Dr F Ramirez from the Brookdale Center for Molecular Biology, Mt Sinai School of Medicine, New York. The cDNA was 3.185 kb which encodes exons 21 to 52 of the human collagen type α1(II). The cDNA was subcloned into EcoR1 site of
35 pBluescriptIIISK (Stratagene). Antisense DIG and radioactively labelled cRNA probes were made by linearising the insert with BamHI and T7 RNA

polymerase. Sense DIG and radioactively labelled cRNA probes were made by linearising the insert with HindIII and using T3 RNA polymerase.

Collagen Type IX (pKTh123)

5 The cDNA was kindly supplied by Dr Y. Ninomiya from the Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA, USA. The cDNA was 0.6 kb which encodes two-thirds of COL2 region through to half of NC2 region of human collagen type alpha1(IX). The cDNA was subcloned into EcoRI site of pBluescript (Stratagene). The insert
10 was linearised with KpnI and T3 RNA polymerase was used to make antisense radioactively labelled cRNA probes.

Collagen Type X (NC1)

15 The cDNA was kindly supplied by Dr J. Bateman from the Department of Paediatrics, University of Melbourne, Victoria, Australia. The cDNA was approximately 0.7 kb which encodes the NC1 domain of human collagen type alpha1(X). The cDNA was inserted into the HindIII/SacI sites of pGEM7Zf(+) (Promega). The template was linearised with HindIII and SP6 RNA polymerase was used to make antisense radioactively labelled cRNA probes.

20

Aggrecan Probe (rpg4.16)

25 The cDNA was obtained from lgt11 library constructed from Swarm rat chondrosarcoma mRNA, kindly supplied by Dr K. Doege from the Research Department, Shriners Hospital, Portland, OR, USA (GenBank accession number J03485). The cDNA was approximately 1.6 kb which encodes the hyaluronic-acid binding region (G1 through half of G2). The cDNA was subcloned into EcoRI site of pBluescript (Stratagene). The insert was linearised with KpnI and T3 RNA polymerase was used to make antisense radioactively labelled cRNA probes.

30

Decorin Probe (P2)

35 The cDNA was kindly supplied by Dr Larry W Fisher from the Bone Research Branch, NIDR, Bethesda, USA. The cDNA was made from mRNA isolated from human bone cells and inserted into the EcoRI site of pBluescript SK (Stratagene). The 1.6 kb insert contained the full sequence for coding human bone decorin. The template was linearised with BamHI and

T7 RNA polymerase was used to make antisense radioactively labelled cRNA probes.

Unknown gene product (DACC7)

5 A hybrid riboprobe (HC22pBluescriptIIISK) was designed to screen a deer antler cDNA library (biased for highly expressed population) for collagen-like and abundantly expressed genes. All screened sequences were identified and sequenced, as described later. BLAST and FASTA analysis identified one unique insert (DACC-7) and found to be approximately 1 kb in
10 length. Gene-specific primers were then designed from this sequence for 5' RACE to obtain the 5' end of the DACC7 gene, which was sequenced and cloned as described later. The full sequence (1.474 kb) for DACC7 in pBK-CMV (Stratagene) was linearised with EcoRI and T7 RNA polymerase was used to make antisense DIG-labelled RNA probes. For sense DIG-labelled
15 cRNA probes, the insert was linearised with XbaI and using T3 RNA polymerase.

In situ Hybridisation

DIG-labelling cRNA probe preparation

20 The DIG-Chem-Link labelling and Detection Set was purchased from Roche (Roche, Australia). The cDNA template was linearised with the appropriate restriction enzyme and 1 µg cDNA template was dried under vacuum. To the dried cDNA template, the following was added: 2 µL of 10 X transcription buffer (400 mM Tris-HCl, pH 8.0; 60 mM MgCl₂; 100 mM
25 dithiothreitol (DTT) and 20 mM spermidine); 13 µL of DEPC-treated water; 2 µL of 2.5 mM Nucleotide mix (10 mM rATP, 10 mM rCTP, 10 mM rGTP, 10 mM rUTP, pH 7.5); 2 µL of appropriate RNA polymerase (T7) and 1 µL of RNase Inhibitor. The mixture was briefly centrifuged then incubated for 2 hours at 37°C. The cDNA template was removed from the mixture after 2
30 hours incubation by directly adding 2 µL of DNaseI I and incubated at 37°C for 15 minutes. *In vitro* transcription was stopped by adding 2 µL of 0.2 M EDTA (pH 8.0) solution. The cRNA probe was then purified using Quick Spin Columns (Roche) as per manufacturer's instructions. The cRNA probe was eluted in 50 µL STE buffer (10 mM Tris, pH 8.0, 1 mM EDTA, 100 mM
35 NaCl). The yield was measured by spectrophotometry, as described

previously. The cRNA probe was then labelled with DIG using the DIG-Chem-Link labelling reagent as per kit instructions and stored at -70°C .

In situ hybridisation

- 5 The HC22 cRNA probe and DACC7 cRNA probe (1.474-kb unique sequence) expression localisation were compared by *in situ* hybridisation. The paraffin embedded tissue sections were deparaffinised in xylene and rehydrated in decreasing concentration ethanol solutions. The slides were immersed into xylene twice for 3 min and twice in 100% ethanol for three
- 10 min. They were placed in 95% ethanol for 3 min and 70% ethanol for 3 min. Finally, the slides were immersed into DEPC-treated water for 3 min to complete rehydrating the tissue sections. The sections were then treated with 200 mM HCl at room temperature for 10 minutes to inactivate endogenous alkaline peroxidase and uncover the RNA from proteins. The slides were
- 15 then washed 5 times in DEPC-treated water to remove the HCl. The sections were then incubated with agitation in 0.25% (v/v) acetic anhydride/0.1 M triethanolamine HCl/0.9 % (w/v) NaCl buffer (pH 8.0) at room temperature for 10 min to bind positively charged molecules and protects RNA. The slides were again washed 5 times in DEPC-treated water to remove the acetic
- 20 anhydride. The slides were initially placed in 95% ethanol, followed by 100% ethanol to dehydrate the tissue sections. Seventy microlitres of standard hybridisation buffer with 50 % formamide (formamide, 50% (v/v); 5 x SSC (0.1 M NaCl, 0.8 M NaCitrate, pH 7.0); 2% blocking reagent (Roche Kit) was placed on the slides to prehybridise at 55°C for 2h in a humid chamber.
- 25 After prehybridisation was complete, 65 μL of standard hybridisation buffer with 50% formamide containing 400 ng/mL of DIG-labelled cRNA probe was denatured at 80°C for 5 min, then placed on the slides with coverslips. The sections were placed in a humid chamber and hybridisation was carried out overnight at 55°C .
- 30 The coverslips were carefully soaked off the slides by soaking for 30 min with 2 X SSC at room temperature. Stringent washes were 55°C for 1h with 2 X SSC, then twice at 55°C for 30 min with 0.1 x SSC. The slides were then equilibrated in TBST (Tris buffered saline with 0.3 % Tween-20 (Sigma), pH 7.5) for 5 min in the Sequenza Immunostaining System (Shandon, UK),
- 35 before incubating in 100 μL of 1:50 diluted antibody conjugate (rabbit F(ab) anti-DIG, alkaline phosphatase-coupled, Dako #D5105) in 0.5% (w/v)

blocking reagent/TBST for 30 min at room temperature. The unbound antibody conjugate was removed by washing 5 min with TBST at room temperature. The slides were then removed from the Sequenza system and a Pap pen (Dako #S2002) was used to create a hydrophobic region around the tissue sections. The colour-substrate solution (5-Bromo-4-Chloro-3-Indoxyl Phosphate (BCIP)/Nitro Blue Tetrazolium Chloride (NBT) (Dako #K0598)) was added to slides to initiate colour development for the desired mRNA signal. The mRNA hybridised with the probe formed purple particles in the tissue sections. After the desired purple dots appeared on the slides and the colour reaction was stopped by washing the slides for 2 min with 50 mL of DEPC-treated water. The slides were then mounted with Aquaperm Mounting Medium (IMMUNON™ Thermo, Shandon, PA, USA), then a coverslip placed with Euckitt (O'Kindler GmbH and Co., Freiberg, Germany) and stored in the dark until analysed.

15

cDNA Expression Library Generation and Screening

cDNA library generation

An amplified lambda cDNA library was prepared from the first antler growth of a 2 year old Red deer stag (*Cervus elaphus*) using the ZAP-cDNA®/Gigapack® III Gold Cloning kit (Stratagene). All reagents were included in the kit unless otherwise stated and the kit protocol was strictly followed. This kit allows construction of directional cDNA libraries, therefore doubles the number of clones detectable by screening. It was designed for optimal library construction, including *in vivo* excision, eliminating subcloning procedures and the high-efficiency lambda system, increasing the size of the library, along with size exclusion providing a true representative cDNA library of the original population of mRNA. The representation has not been altered by PCR amplification, and only a single amplification of the library was performed. In brief, 375 µg of total RNA was extracted from the deer antler, devoid of skin, as described previously. A total of 5.175 µg of polyA RNA was extracted from this total RNA sample using a Dynabeads® mRNA Purification kit (DynaL Pty Ltd, Carlton South, Victoria, Australia). This kit purifies polyA RNA from total RNA using oligo (dT)₂₅ magnetic beads, so that ribosomal and transfer RNA were not included in the library. First strand cDNA was synthesised from this mRNA using the ZAP-cDNA® Synthesis kit (Stratagene). The double stranded cDNA was

ligated into the lambda Uni-ZAP® XR vector using EcoRI (5' end) and XhoI (3' end) sites. This vector accommodates a DNA insert up to 10 kb in length.

The lambda library was then packaged using the Gigapack® III Gold Cloning kit (Stratagene) and the packaged recombinant lambda phage plated using the *E. coli* cell line XL1-Blue MRF'. At this stage, titring of the primary library identified a recombinant titre of 7.98×10^7 plaque forming units per μg vector arms. As primary libraries can be unstable, the library was amplified to obtain a more stable, higher titre stock. The amplified library titred at $1.308 \times 10^9/\text{mL}$. Detailed methodologies can be obtained from both the ZAP-cDNA® Synthesis kit and the Gigapack® III Gold Cloning kit (Stratagene).

Preparation of cDNA Library Filters for Screening

For screening the cDNA library, large 135 mm plates were used to achieve approximately 50,000 plaques per plate. Once the plaques had formed, the plates were maintained at 4°C for approximately 2 h to allow the top agarose to harden before filter lifting. Colony/Plaque Screen™ membranes (DuPont) were labelled on the tab and placed face down on the plate for 2 min, during which time the orientation holes were marked on the bottom of the plate. When duplicate plaque lifts were performed, then the second filter was in contact with the plate for a duration of 5 min to allow efficient transfer. Using a plate lid as a dish, 3MM paper covering the bottom was saturated with a denaturing solution (1.5 M NaCl, 0.5 M NaOH) and the filter was placed plaque side up in this solution for 2 min. The filter was then dragged along the lip of the tray to remove excess solution and placed in a second tray saturated with a neutralising solution (1.5 M NaCl; 0.5 M Tris HCl, pH 8.0) for 5 min. This process was repeated with a rinse solution (0.2 M Tris HCl, pH 7.5; 2 X SSC) for 30 sec. The filter was then blotted between sheets of 3MM paper and exposed to an energy level of 120 mJ in a UV Stratalinker 1800 (Stratagene) to crosslink the DNA to the filter. While still moist, the filter was sealed in a plastic bag and stored until ready to hybridise.

Screening a cDNA library with riboprobes

cDNA filters were prehybridised either back to back or between mesh in the hybridisation bottle system (Hybaid) when more than 2 filters screened

at one time. Prehybridisation, hybridisation and washing were performed as for Northern blot hybridisation as described previously.

Hybridisation marks on the filter image corresponding to plaques were cored and a secondary screening was performed. Clones surviving the second screening underwent a final tertiary screening before consideration for further characterisation. Any clones that survived this screening procedure were *in vivo* excised.

In vivo excision

The design of the Uni-ZAP[®] XR vector (Stratagene) allowed *in vivo* excision and recirculation of the cloned cDNA insert contained within the lambda vector arms to form a plasmid containing the cloned insert. As a plasmid, the cloned cDNA could be stored as a glycerol stock, and mini DNA preparations could be performed. Thus, any cored plaque of interest was *in vivo* excised for further characterisation. The methodology for *in vivo* excision could be found in the protocol for the ZAP-cDNA[®] Synthesis kit (Stratagene) which also contained the required reagents.

Mini-preparation of plasmid DNA

Mini-preparation of plasmid DNA was prepared by using the Wizard[®] Plus Miniprep DNA Purification system (Promega) as described previously. Because of the poor yields of the plasmid DNA it was necessary to transform the plasmid into another host, DH5 α , to obtain better quality DNA (as described later). Clones were sequenced and also reassessed using PCR techniques to help characterise the clones (as described later). Clones of interest were selected for midiprep DNA extraction as described previously.

5' RACE (Rapid Amplification of cDNA ends), cloning and sequencing ***5' RACE***

5' RACE is a procedure for amplification of nucleic acid sequences from a messenger RNA template, between a defined internal site and unknown sequences at the 5' end of the mRNA. This technique was used to obtain the 5' end of the DACC7 gene using sequence information provided from the partial 3' DACC7 clone obtained from screening of the cDNA library to generate DACC7GSP1 and nested DACC7GSP2 gene-specific primers for 5' RACE.

A 5' RACE System of Rapid Amplification of cDNA ends, Version 2.0 kit (Gibco BRL, Life Technologies) using 4 protocols was applied on 2 year old Red deer stag DAC RNA as per kit instructions. Gene specific primer 1s' (GSP1s) were designed based on kit instructions and the 5' end of the cDNA library clone sequences. For example, DACC-7 GSP1 (primer for 1st strand synthesis), was a 20-mer with a melting temperature of 63 °C and consisted of 5' GTT CCA CAC GTC ACC ACA GT 3' (SEQ ID NO: 39).

Advantage[®] cDNA PCR kit (CLONTECH Laboratories, Inc., USA) was used in Protocol 4 of the 5' RACE System using the following cycles: 94 °C for 1 min; a step cycle of 94 °C for 0.5 min, 60 °C for 1 min and 72 °C for 5 min for 35 cycles; followed by 72 °C for 7 min to allow final extension. The Abridged Anchor Primer and a nested GSP2 were used in the PCR. GSP2s were nested primers in reference to the GSP1s, designed from the cDNA library clone sequences as per kit instructions. For example, DACC-7 GSP2 (primer for PCR) was a 24-mer with a melting temperature of 60 °C and consisted of 5' CGT ATC GTG CTT AAA TAT GTC AGT 3' (SEQ ID NO: 40).

Cloning Techniques

Cloning of PCR products

Cloning of PCR products into pGEM-T Easy[®] Vector was achieved using a pGEM-T Easy[®] Vector Systems Kit (Promega). A 1:1 insert: vector molar ratio was used and the ligation reaction was incubated at 4 °C overnight as kit instructions.

Transformation of cloned PCR products into JM109 competent cells

The cloned PCR products were transformed into JM109 High efficiency competent cells (Promega) as per kit instructions.

Restriction cloning of DNA

Non-PCR DNA products to be cloned were restriction digested with appropriate enzymes to create overhanging "sticky ends" that were compatible with overhanging ends of similar digested vector. Each restriction digest was gel purified before undergoing the ligation reaction.

For each insert to be ligated, both 1:1 and 3:1 insert:vector molar ratio reactions were carried out using the formula:

$$\frac{\text{ng of vector} \times \text{size (kb) of insert}}{\text{insert:vector molar ratio}} = \text{ng of insert}$$

size (kb) of vector

For every 10 µl of reaction, 1 µL of 10 x ligation buffer (Roche Molecular Systems), 1 µL of T4 DNA ligase (Roche Molecular Systems) and 1 µL of glycogen (which increases the probability of T4 ligase molecules coming into
5 contact with overhanging DNA ends) were combined with the appropriate amounts of vector and insert in 0.5 mL microcentrifuge tubes. After brief mixing and spinning in a microfuge the ligation reaction was incubated at 4°C overnight.

10 ***Preparation of competent cells***

To prepare competent cells for transformation, 50 mL of Luria-Bertaini (LB) medium (10 g tryptone, 5 g yeast extract, 10 g NaCl) was inoculated with 0.5 - 1.0 ml overnight culture (DH5α *E.coli* strain) in a 250 mL conical flask and cultured for 3 - 4 h at 37°C with shaking until the OD₆₀₀ reached 0.5. The
15 cells were chilled on ice for 20 min before spinning at 3,000 rpm for 10 min to pellet the cells. 5 mL cold (4°C) CaCl₂ was added to resuspend the bacteria. The cells could be used immediately for transformation or aliquoted and stored at -70°C.

20 ***Transformation of ligation reactions by electroporation***

For each transformation reaction, 2 µL of overnight ligation reaction was combined with DH5α competent cells in a sterile 1.5 mL microtube and mixed by flicking. The mixture was incubated on ice for 20 min, after which they were heat-shocked at 42 °C in a water bath for 45 s and immediately
25 returned to ice for 2 min. LB medium was added to the tubes and incubated at 37 °C with shaking for 1.5 h. The transformations were then plated onto LB plates containing 100 µg/mL ampicillin and incubated at 37 °C overnight.

Cloning of a putative full length DACC7

30 The expression vector pBK-CMV (Stratagene) is a useful vector for recombinant protein expression. The vector allows expression in both eukaryotic and prokaryotic systems. Eukaryotic expression is driven by the cytomegalovirus (CMV) immediate early promoter. Stable clone selection in eukaryotic cells is made possible with G418 by the presence of the neo-
35 mycin- and kanamycin- resistance gene, which is driven by the SV40 early

promoter with thymidine kinase (TK) transcription termination and polyadenylation signals.

The expression vector pBK-CMV was modified to remove the prokaryotic *lac* promoter and *lacZ* translation start site, since this results in increased eukaryotic expression, essential for protein function studies. This construct was named pBK-CMV.2. A full length DACC7 contig was pieced together using carefully chosen restriction enzymes as outlined in Figure 27 using methods described previously. This was cloned into pBK-CMV.2 but was not fused in frame to the *lacZ* gene and contained non-coding 3' end sequence. The cloning steps and transformations were carried out as outlined previously into DH5 α or JM109 competent cells. To generate plasmid DNA after each cloning step, a miniprep was carried out and restriction digested to obtain plasmid DNA for the next cloning step.

15 ***Sequencing of DNA***

Miniprep plasmid preparations (described previously) of cloned PCR products were sequenced using T7 and SP6 primers. Sequencing was done by AGRF.

20 **Histochemical and immunohistochemical methods**

Histological Staining

Deer antler cartilaginous tips were divided into the 3 zones shown in Figure 21 and each zone subdivided into two equal parts. One-half was immediately fixed in 10% neutral buffered formalin, the other in Histochoice fixative. The fixed tissues were embedded in paraffin and 5 μ m histological sections cut and mounted using standard techniques. The formalin-fixed sections were processed and stained with haematoxylin and eosin or 1% (w/v) Toluidine Blue at pH 1.0 and 2.5 respectively, then counter-stained with fast red dye, as described in detail by Little et al. (1997).

30

Immunohistochemical Staining

For these studies the antler tissues fixed in Histochoice (Amresco #H102-IL, OH, USA) were used. Histochoice is a fixative which does not contain formaldehyde, thereby eliminating the need for recovery of the target and predigestion of paraffin sections. The immunolocalisation of type II collagen was undertaken essentially as described previously (Little et al.

35

1997) but with the following modification. Glass mounted cut sections were incubated at 4°C for 16 h and treated with a commercially available monoclonal antibody (Anti-human type II collagen, purified mouse IgG1, Clone: II-4C11, titre: 500 µg/mL, 1:50 dilution (ICN Biomedicals, OH, USA)).

- 5 A biotinylated secondary antibody (anti-mouse/rabbit immunoglobulin (Dako LSAB₂, K1015) was added for 30 min at 20°C then peroxidase-labelled streptavidin (Dako LSAB + peroxidase K0690) for 30 min at 20°C. Staining was completed following incubation with Nova Red (Vector Laboratory SK-4800) substrate solution and rinsing.

10

Tissue and Cell Preparations for cell culture studies

Deer Antler Cartilage (DAC) Used for Alginate Bead Cultures

- The cartilaginous tips from 3 mature fallow deer stags (*Dama dama*, designated F1, F2, F3) were collected during the maximal growth period under local anaesthetic (Lignocaine) as described previously for RNA preparation. A section of the cartilage centre was removed for histological examination as shown in Figure 3. The remaining deer antler cartilage (DAC) was separated into 3 zones (A, B, C) as shown in Figure 3, corresponding to the prechondrocytes region (zone A), mature proliferating chondrocyte region (zone B), and hypertrophic chondrocyte region (zone C). The predominant chondrocyte populate in these zones were confirmed by the corresponding histological assessment. The DAC zones were discernible morphologically as the prechondrocyte tissue which was observed as a white, soft cartilage with no blood vessels; the mature chondrocyte tissue observed as soft cartilage with blood vessels; and the hypertrophic chondrocyte tissue which showed encroaching mineralisation and blood vessels invasion. Since the 3 zones merged with each, pure cell population from each could not be obtained. The outer rim of cartilage in each DAC zone was discarded, DAC cells from the 3 zones (A, B, and C) were released by enzymatic digestion as described previously for RNA preparation. Their viability was determined by dye exclusion using a haemocytometer.
- 15
20
25
30

DAC Used for Monolayer Cultures

- Antler specimens were collected from 2 fallow deer (F4, F5) and red deer (*Cervus elaphus*) designated deer 6 - antler 1 (R6.1), red deer 6 - antler 2
- 35

(R6.2). Tips of these specimens were dissected as shown in Figure 3 and cells released as described previously.

Sheep Articular Chondrocytes (SAC) Used for Monolayer Cultures

5 Sheep articular chondrocytes (SAC) were obtained from the stifle joints of 4-year-old purebred Merino sheep. Joints were transported to the laboratories on ice within 4 h of sacrifice, were opened under sterile laboratory conditions and full-depth articular cartilage was sliced from the tibial plateaux (TP) and the femoral condyles (FC) including the trochlear
10 groove using a #11 blade. Each cartilage area (TP or FC) was enzyme digested with 0.1% (w/v) pronase (Boehringer Mannheim Australia Pty. Ltd., Castle Hill, NSW, Australia) in DMEM:F12 media containing 10% (v/v) FBS at 37°C for 2 h then changed to 0.04% (w/v) collagenase in DMEM:F12/10% (v/v) FBS for digestion overnight at 37°C to release the cells. Cells were collected
15 through a sterile 70µm cell strainer and viability determined by dye exclusion using a haemocytometer.

Rabbit Ear Chondrocytes (REC) Used for Monolayer Cultures

20 Rabbit ears were dissected from a New Zealand male rabbits and cartilage obtained by meticulously removing the skin and periosteum under sterile conditions in a laminar flow cabinet. The diced ear cartilage was enzyme digested with 0.125% (w/v) trypsin in DMEM:F12 at 4°C overnight, then 37°C for 1 h. This was replaced with media containing 0.04% (w/v) collagenase and supplemented with 10% (v/v) FBS at 37°C for 5 h, vortexing
25 for 10 sec every 30 min. REC were collected through a sterile 70µm cell strainer and viability determined by dye exclusion using a haemocytometer.

REC Used for Explant Cultures

30 REC was collected as described above, except that there was no digestion step, instead the prepared cartilage was diced into explants (approx. 1mm²) and used directly for culture experiments.

Cell Culture Methods

DAC Alginate Bead Cultures

35 DAC bead cultures were prepared essentially as described by Häuselmann *et al.* (1994). Briefly, for each zone (A, B, C) DAC cells obtained

- after collagenase digestion were centrifuged and washed twice with DMEM:F12. The cell pellets were re-suspended at a density of 3×10^6 cells/mL in alginate solution which contains 1.2% (w/v) sodium alginate (Sigma) dissolved in 0.15M NaCl (Ajax Chemicals, Auburn, NSW, Australia).
- 5 The cell suspension was slowly expressed through a 23-gauge needle and the droplets formed allowed to fall into a 100mM CaCl_2 (May and Baker Australia Pty. Ltd., Australia) solution. The beads (20,000 cells/bead) were allowed to polymerise in this solution for 10 min. They were then transferred to a 48 (Costar, Cambridge, MA, USA), (10 beads/well) or 96 (Greiner,
- 10 Maybachstrasse, Frickenhausen, Germany), (2 beads/well) well plates and covered with DMEM:F12/10% (v/v) FBS medium. After 24 h incubation at 37°C in an atmosphere of 5% CO_2 /95% air with 75% humidity, DAC conditioned media (DAC-CM) was collected from each well.

15 *DAC Monolayer Cultures*

- DAC cells prepared as described previously were seeded into 75cm^2 flasks culture flasks at 2×10^6 cells/mL by incubating in DMEM:F12 media with and without 10% FBS at 37°C in an atmosphere of 5% CO_2 /95% air with 75% humidity. DAC CM was collected from each primary culture (i.e. media
- 20 was replaced but the cells were not subcultured) at specified time points. DAC-CM samples were prepared from specimens F4, F5, R6.1 and R6.2 and collected on days 1, 3, 5, 7, 9, 11, 13 and 18 post-culture initiation.

SAC Monolayer Cultures

- 25 SAC were cultured as monolayers at 1×10^5 cells/mL in 75cm^2 flasks (Corning) with DMEM:F12 media containing 10% FBS at 37°C in an atmosphere of 5% CO_2 /95% air with 75% humidity. Once confluence was reached, SAC were treated with various concentrations of DAC-CM obtained from DAC bead culture experiments from zones A, B and C collected after
- 30 24 h. DAC-CM concentrations used were 1, 3, 10, 30, 100% (v/v) or control media [DMEM:F12/10% (v/v) FBS]. These experiments were used to determine DAC cell zonal synthesis of DNA and total proteoglycan (PG) synthesis.

35 *REC Monolayer Cultures*

REC were cultured as monolayers at 5×10^4 cells/ml in 75cm² culture flasks with DMEM:F12 media containing 10% (v/v) FBS at 37°C in an atmosphere of 5% CO₂/95% air with 75% humidity. Once confluence was reached, REC were treated with 50% DAC-CM from DAC bead or monolayer cultures, i.e. A, B and C collected after 24 h and F4, F5, R6.1, R6.2 media collected 1 d, 3 d, 5 d, 7 d, 9 d, 11 d, 13 d and 18 d post-culture initiation.

10 *REC Explant Cultures*

Diced (~ 1 mm x 1 mm) explants of REC were cultured (4 explants/well) with DMEM:F12 media containing 10% (v/v) FBS at 37°C in an atmosphere of 5% CO₂/95% air with 75% humidity. The media was removed and REC cells were treated with DAC-CM from DAC bead culture obtained from regions A, B and C per 1 d or from CM from cultures from F4, F5, R6.1, R6.2 collected at 1 d, 3 d, 5 d, 7 d, 9 d, 11 d, 13 d and 18 d post-culture initiation.

Mouse Fibroblast Cell Line

A 3T3 Swiss Albino P137 contact inhibited cell line (CSL, Victoria, Australia, ATCC CCL 92) was used for the growth factor assay, as described by Klagsburn *et al.* (1977). 3T3 cells were cultured in 96-well plates (5×10^4 cells/mL, 1×10^4 cells/well) in DMEM:F12/10% (v/v) FBS at 37°C in an atmosphere of 5% CO₂/95% air with 75% humidity. The media was removed and 3T3 cells were treated with DAC-CM from DAC bead culture obtained from regions A, B and C per 1 d or from CM from cultures from F4, F5, R6.1, R6.2 collected at 1 d, 3 d, 5 d, 7 d, 9 d, 11 d, 13 d and 18 d post-culture initiation.

30 *Assay for Biosynthesis of Proteoglycans*

DAC Alginate Bead Cultures

Alginate beads from each DAC zone (A, B and C) were placed in 48-well plates (10 bead/well) and incubated with DMEM:F12 media containing Na₂³⁵SO₄ (Amersham, Cardiff, UK) added (5μCi/well) for 8 h, 24 h, 48 h and 72 h. At the termination of the incubations media and alginate beads were processed separately (4 replicates) at each time-period. Alginate beads and

their respective media were individually digested with papain (Sigma) (50 μ g/mL in PBS containing 10mM EDTA and 5mM cysteine) at 60°C for 2 h and unincorporated $^{35}\text{SO}_4$ was removed using BaSO_4 precipitation as previously described by Collier and Ghosh (1989). Briefly, an aliquot of the
5 papain digested sample (400 μ L) was mixed with a solution of 0.1M Na_2SO_4 containing 25mg/mL chondroitin sulphate (Sigma Chemical Co.) (200 μ L). To this solution was added 100 μ L of 0.4M BaCl_2 . The sample was vortexed, centrifuged (2500 x g) and a 400 μ L aliquot of the supernatant collected and the above described precipitation repeated. A 250 μ L aliquot of the
10 supernatant from the second precipitation was collected and 50 μ L of 1.1mg/mL chondroitin sulphate in 0.3M BaCl_2 added. The sample was vortexed, 50 μ L of 0.2M Na_2SO_4 solution was added and the sample vortexed again and centrifuged as before. A 100 μ L aliquot of this supernatant was collected, mixed with 5mL scintillant (Emulsifer Safe®, Canberra Packard,
15 Gladesville, NSW, Australia) and the radioactivity determined by liquid scintillation spectrophotometry (Model 1500 Liquid Scintillation Analyser, Canberra Packard) and the disintegrations per minute (DPM) determined for 2 min. The DPM of each 100 μ L sample were multiplied by the dilution factors inherent in the assay to give a total DPM per sample.

20

Effects of DAC-CM on SAC Synthesis of Proteoglycans in Monolayer Cultures

Cells isolated from the TP or FC of sheep joints were cultured in 24-well plates (Nunc, Denmark), 60,000 cells/well. DAC-CM from zones A, B or
25 C region at concentrations 1, 3, 10, 30, 50 and 100% (v/v) or control (DMEM:F12/10% (v/v) FBS) all containing $\text{Na}_2^{35}\text{SO}_4$ (5 μ Ci/well) were added to the wells. After 48 h incubation the media and cells were collected separately, papain digested and ^{35}S -labelled PGs isolated and counted, as described previously.

30

Effects of DAC-CM on REC Synthesis of Proteoglycans in Explant Cultures

REC explants were placed in 24-well plates (4 explants/well). To some wells DAC-CM diluted to a concentration of 50% (v/v) with DMEM:F12/10% (v/v) FBS, and $\text{Na}_2^{35}\text{SO}_4$ (5 μ Ci/well) were added. Control wells contained only
35 DMEM:F12/10% FBS and $\text{Na}_2^{35}\text{SO}_4$ (5 μ Ci/well). After 48 h incubation the

media and explants were collected separately, papain digested and ^{35}S -labelled PGs isolated and counted as described previously.

Assay for DNA Synthesis

5 **DAC Alginate Bead Cultures**

DNA synthesis of DAC cells in alginate beads were determined using the assay described by Hutadilok *et al.* (1991) with the modification that the beads were dissolved as described by Häuselmann *et al.* (1994). Briefly, for each DAC zone (A, B, C), alginate beads (2 beads/well) were placed in 96-well
10 plates. After 24 h incubation, media was changed and ^3H -thymidine added ($0.5\mu\text{Ci/well}$). After 8, 24, 48 and 72 h incubation with ^3H -thymidine (5 replicates), media was discarded, beads dissolved in NaCl (Häuselmann *et al.* 1994) and cells collected using a cell harvester (Titertek Plus) onto glass filter paper (ICN Biomedicals, Costa Mesa, Ca, USA). The incorporated
15 radioactivity into DNA was determined by liquid scintillation spectrophotometry (Model 1500 Liquid Scintillation Analyser) by mixing 3mL scintillant with the glass filter paper and DPM counted for 2 min. Results were expressed as DPM/well (mean \pm sem).

20 **SAC Monolayer Cultures**

SAC from the TP or FC were cultured in 96-well plates (15,000 cells/well). DAC-CM from zones A, B or C region at concentrations 1, 3, 10, 30, 50 and 100% (v/v) or controls containing no DAC-CM [DMEM:F12/10% (v/v) FBS] plus ^3H -thymidine ($0.5\mu\text{Ci/well}$) were added to each well. After
25 24 h incubation, ^3H -thymidine-labelled DNA was determined as described previously.

REC Monolayer Cultures

REC were cultured in 96-well plates (10,000 cells/well) with media
30 containing DAC-CM at 50% (v/v) concentration or controls containing no DAC-CM [DMEM:F12/10% (v/v) FBS] plus ^3H -thymidine ($0.5\mu\text{Ci/well}$). After 24 h incubation, ^3H -thymidine-labelled DNA was determined as described previously.

35 **3T3 Mouse Fibroblast Cultures**

3T3 cells were incubated with media containing DAC-CM from zones A, B or C at 50% (v/v) concentration or with control containing no DAC-CM [DMEM:F12/10% (v/v) FBS]. ^3H -thymidine ($0.25\mu\text{Ci/well}$) was added to each well and after 3 h incubation, media was removed, cells were harvested and ^3H -thymidine-labelled DNA levels determined as described previously.

Metabolic Activity of Cells Using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) Assay

SAC Monolayer Cultures

Cellular metabolism of SAC (mitochondrial dehydrogenase activity) in the absence and presence of DAC-CM was determined using the assay method described by Mosmann (1983) but with the following modifications: SAC TP or FC were incubated for 24 h in 96-well (15,000 cells/well) with DMEM:F12/10% FBS or 1, 3, 10, 30, 50 and 100% (v/v) of DAC-CM from each zone. MTT ($10\mu\text{L}$, 5mg/mL in PBS) was added to each well and the plates were incubated for a further 2 h at 37°C . Media was removed and $100\mu\text{L}$ (w/v) SDS in 55mM Na-citrate/ 150mM NaCl was added to each well to dissolve the crystals. Colour development in wells was then read in the Thermomax microplate reader (Molecular Devices, Menlo Park, Ca, USA) set at a wavelength of 562nm .

Statistics

The Student's t-Test was used to determine whether two means from individual samples were significantly different, where $p < 0.05$.

Proteomics

This part of the analysis was facilitated by access to the Australian Proteome Analysis Facility established under the Australian Government's Major National Research at Macquarie University.

Samples of conditioned media from alginate bead cultures from antler of F4 and F5 fallow deer were collected at 24 h and 7 d (168 h) after initiation of cultures. Each supernatant sample was submitted to amino acid analysis to determine the protein content of each sample. This analysis showed that sample 1 (F4 – 24 h) had 1.49mg/mL , sample 2 (F4 – 168 h) had 1.14mg/mL , sample 3 (F5 – 24 h) had 1.15mg/mL and sample 4 (F5 – 168 h) had 0.61mg/mL of protein. Samples underwent TCA precipitation to purify proteins, then

were solubilised with sonication for 30 s. Endonuclease was added and samples were then centrifuged at 20,000 x g for 10 min. Samples were then loaded onto gels for Isoelectric Focusing (IEF). For the range pH3-6 and pH5-8 gradient strips were loaded via in-gel rehydration; for pH6-11 gradient strips were cup loaded at the anode. For first dimension IEF, 95,000Vh separating gel gradient 8-18%T large format polyacrylamide slab gels were used, while for second dimension electrophoresis, 6 h @ 3mA/gel 14 h @ 15mA/gel conditions were employed. Gels were stained with SYPRO Ruby fluorescent stain, scanned to produce a digital image and the resultant sample images were compared using Z3 Image Analysis Software (Compugen). The triplicate images from each of the culture supernatants were used to compile a raw master reference gel composite. The 3 composite gels generated for each sample were then used to compare protein profiles between culture supernatants. This was done for pH3-6, pH5-8 and pH6-11 gradients. The acquired image analysis data was then used to identify potential targets for a 16 h protein tryptic digest at 37°C. The resulting peptides were purified using a ZipTip to concentrate and desalt the sample. The samples were then analysed by ESI-TOF MS/MS using a Micromass Q-TOF MS equipped with a nanospray source and data manually acquired using borosilicate capillaries. Data was acquired over the m/z range 400-1800 to select peptides for MS/MS analysis. After peptides were selected, the MS was switched to MS/MS mode and data collected over the m/z range 50-2000 with variable collision energy settings.

25. **RESULTS**

cDNA Sequences Overexpressed in Antler Cartilage Cells

The present invention is based on the unexpected and surprising discovery that chondrocytes of rapidly growing cartilage of regenerating deer antler express unique genes products which are not expressed in articular cartilage or epiphyseal growth plate chondrocytes of adult or full-term foetal deer, ovine or human cartilages. Of even greater surprise is the finding that several of these gene transcripts, seen as separate band on Northern Blot analysis, are only expressed in the early stage of chondrogenesis in human foetal tissues.

35 In the use of various collagens as antigens for treatment of arthritic diseases by oral tolerance which is mediated by T-cells, type II collagen from

bovine origins is known to be less effective than type II collagen derived from the chick (Cremer et al. 1992; Zhang et al. 1990; Hart et al. 1993; Myers et al. 1993; Weiner et al. 1994; Barnett et al. 1996; Trentham et al. 1993; Sieper et al. 1996).

5 The present invention has demonstrated the cloning of a novel gene using deer antler cartilage as the starting material. A full-length clone was obtained by screening a cDNA library and by applying the technique of 5' RACE. The pattern of expression of the gene was examined in human tissues at the mRNA level and the human chromosomal localisation of this novel
10 gene was also established.

A lambda phage library containing clones ligated via EcoRI and XhoI ends in lambda Uni-ZAP XR (as described previously) was made from deer antler cartilage (DAC). This DAC cDNA library was screened for highly expressed cDNAs. Starting probe material for screening the deer antler
15 cartilage cDNA library was generated by creating a hybrid cDNA template consisting of pBluescriptSK and a partial collagen sequence (HC22). This cDNA template was used to make a ³²P radiolabelled RNA probe. The library was screened using this probe as described previously. After primary screening, 15 clones were selected as positive by identification of
20 corresponding radioactive dots on the phosphorimager. After secondary screening, 14 out of the original 15 remained positive. Tertiary screening confirmed that all 14 clones from secondary screening were positive, and single isolated positive phage clones were selected for in vivo excision to release the plasmid. Plasmid DNA was then obtained by miniprep.

25 The miniprep DNA was digested with EcoRI and XhoI to release the inserts and run on a 1% agarose gel. The clones were found to range in size between 0.5 kb and 1.5 kb. These were sequenced for further identification using universal forward (5' sequencing primer) and reverse (3' sequencing primer) primers. Sequence homology analysis revealed that the 14 clones
30 could be grouped into 9 clusters (as shown in Figure 5), representing homology with Human α 1 type II collagen, Human prepro-alpha1 (I) collagen, Human procollagen alpha 2(V), Human KIAA1075 protein (tensin2), Human SPARC/osteonectin, Human ribosomal protein S2 (RPS2), Human ribosomal protein L23a, Human non-histone chromosomal protein (HMG-14), and
35 Human LOC133957 protein of unknown function (Genbank BC015349).

Because the SOLR *E. coli* host yielded low quantities of plasmid DNA, DAC clones were transformed into *E. coli* DH5 α host, which was a better host for plasmid DNA production. The 14 clones were fully sequenced (except for DACC-9) by primer walking and open reading frames were found.

5

DACC-7

Sequence alignments with human LOC133957 and mouse RIKEN 0610011N22 (Genbank BC003345, of unknown function) showed that the DACC-7 clone was very unlikely to be full length based on comparison of size
10 and sequence.

Using the technique of 5' RACE as described previously, the 5' end of DACC-7 was obtained. RNA was made from deer antler cartilage tissue and checked for integrity on a denaturing agarose gel. A 5' RACE kit (CLONTECH) was used on deer antler cartilage RNA (as described previously)
15 in attempt to obtain 5' DACC-7. The primers used for 5' RACE were made based on sequence information from the 5' end of the DACC-7 deer antler cartilage library clone as shown in Figure 5. After the first round of PCR amplification, only a faint band could be seen in each lane. Gel purification and a second round of PCR were necessary to see clearer bands. The sizes of
20 these broad bands were approximately 0.6 kb but were difficult to determine at this stage. After further gel purification, the PCR products were cloned into pGEM T-Easy[®] vector (Promega) as described in the methods and transformed into high efficiency competent *E. coli* cells JM109 (Promega). Plasmid DNA was isolated by miniprep, digested with EcoRI to release the
25 insert and run on a 1% gel. One distinct insert was identified, approximately 0.7 kb in size. A midiprep plasmid preparation of this product was sequenced using universal forward (T7) and reverse (SP6) primers. The sequencing information showed that the 0.7 kb RACE product was determined to be 0.729 kb by sequencing. This overlapped 0.287 kb of the
30 DACC7 deer antler cartilage library clone and extended the sequence 5' by 0.442 kb, making DACC-7 gene product approximately 1.5 kb in length. The first methionine (ATG) start site that produced the longest open reading frame was 48 bp from the 5' end of the DACC-7 gene product. Thus a 5' untranslated region of 47 bp was identified.

35

Generating a full length DACC-7 construct in a eukaryotic expression vector, such as pBK-CMV, was necessary for future expression of the DACC-7

protein. Using suitably located single-cut restriction enzymes and a procedure as described in the methods (Figure 27), the 0.7 kb RACE product and the library clone were joined together to generate a putative DACC-7 cDNA of 1.5 kb (including 5' untranslated region) with an open reading frame of 258 aa. Directional cloning using non-complementary sticky ends ensured correct orientation of each insert.

A contig of the sequences of the 0.7 kb RACE product and the 1 kb DACC-7 library clone was analysed for amino acid sequence homology with human (LOC133957) and mouse (RIKEN 0610011N22) homologs (Figure 15). Comparison of the sizes and sequences of DACC-7 with human (LOC133957) ortholog strongly suggest that the DACC-7 contig is full length. A website called NCBI Entrez Genome map view (<http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps>), which provides information on gene clusters localised to the human genome, has a chromosomal localisation of the human LOC133957 gene – Chr.5, gi|17444086: 171999-185824. This location corresponds to the region 5p15.33. The 1.5 kb full length DACC-7 cDNA contains an open reading frame of 0.777 kb (258 aa) that is shorter than the human (LOC133957, 0.783 kb, 260 aa) or mouse (RIKEN 0610011N22, 0.783 kb, 260 aa) homologs, with 2 deleted amino acids at the 3' end (131aa and 132aa). Comparison of the DACC-7 open reading frame with human (LOC133957) and mouse (RIKEN 0610011N22) homolog sequence has shown that the DACC-7 sequence obtained is very likely to be full length. As shown in Figure 15, there is a reasonably high homology of DACC-7 with human LOC133957 and mouse RIKEN 0610011N22, demonstrating that these are species homologs of DACC-7.

Examination of the DACC7 amino acid sequence revealed that DACC-7 sequence had potential a N-glycosylation site (N-X-S or N-X-T where X is any amino acid except proline) at 98aa...100aa. Based on the amino acid usage (Figure 15), the polypeptide backbone of the DACC-7 protein was predicted to be 30kDa. The presence of a N-glycosylation site suggests the size of DACC-7 protein to be larger *in vivo*. A signal peptide was detected by SMART database (identifies domains, <http://smart.embl-heidelberg.de/>) at 1aa...46aa and is thus likely to be a secreted protein, directed out of the cell. The DACC-7 protein was determined to be a basic protein from the pI value (Figure 15). Thus DACC-7 protein could potentially bind to proteoglycans, a

major constituent of the extracellular matrix (a negatively charged environment).

DACC-2

5 The polypeptide sequence encoded by this cDNA sequence shares up to 98% sequence identity with known vertebrate collagen alpha 1(II) chain precursors which includes human (Su et al. 1989: Accession No. P02458) and mouse sequences (Metsaranta et al., 1991: Accession No. B41182). Type II collagen fibrils are known as a major structural protein forming extracellular
10 matrix structures of connective tissues, such as cartilage, nucleus pulposus and vitreous body. It maintain the shape and to resist the deformation of the tissues.

 The most closely relate gene family to vertebrate collagen alpha 1(II) chain precursors are Type I collagen which are approximately 68% identical
15 the polypeptide sequence encoded by DACC-2.

DACC-3

 The polypeptide sequence encoded by this cDNA sequence shares up to 98% sequence identity with known 40S ribosomal protein S2(S4) (LLREP3
20 protein) which includes human (Slynn et al. 1990: Accession No. P15880) and mouse sequences (Heller et al. 1988: Accession No. P25444). RPS2 is known to function as both a ribosomal protein (component of the 40S subunit) for mRNA binding and is required during oogenesis (as demonstrated by a sterile female RPS2 mutant fly model).

25 The most closely relate gene family to vertebrate 40S ribosomal protein S2(S4) is the human ortholog of the mouse wisZ protein which is approximately 76% identical the polypeptide sequence encoded by DACC-3.

DACC-4

30 The polypeptide sequence encoded by this cDNA sequence shares up to 100% sequence identity with known ribosomal protein L23a which includes human (Wool et al. 1995: Accession No. NP_000975) and rat sequences (Suzuki and Wool, 1993: Accession No. CAA46336). L23a is a ribosomal protein that is a component of the 60S subunit. The protein may
35 be one of the target molecules involved in mediating growth inhibition by interferon.

The most closely relate gene family to vertebrate ribosomal protein L23a is the 60S ribosomal protein which is approximately 83% identical the polypeptide sequence encoded by DACC-4.

5 DACC-5

The polypeptide sequence encoded by this cDNA sequence shares up to 81% sequence identity with known human high-mobility group (non-histone chromosomal) protein 14 (Accession No. XP_049753). HMG-14 which binds to the inner side of the nucleosomal DNA, potentially altering
10 the interaction between the DNA and the histone octamer. Like HMG-14, it may be involved in the process that maintains transcribable genes in a unique chromatin conformation.

DACC-6

15 The polypeptide sequence encoded by this cDNA sequence shares up to 98% sequence identity with tensin2 (Accession No. XP_029631). Tensin2 positively regulates cell migration. The tensin family role is in regulating cell motility.

The most closely relate gene family to this protein is tensin which is
20 approximately 65% identical the polypeptide sequence encoded by DACC-4.

DACC-8

DACC-8 appears to be non-coding, however, shares a high degree of sequence identity the mRNA encoding osteonectin (Lankat-Buttgereit et al.,
25 1988). Osteonectin appears to regulate cell growth through interactions with the extracellular matrix and cytokines. Osteonectin binds calcium and copper, several types of collagen, albumin, thrombospondin, PDGF and cell membranes. Osteonectin is expressed at high levels in tissues undergoing morphogenesis, remodelling and wound repair.

30 The most closely relate gene family to vertebrate osteonectin is the human SPARC-like 1 protein which is approximately 57% identical to human osteonectin.

DACC-9

35 Two sequences were obtained, one to the 5' end and another to the 3' end. The polypeptide sequence encoded by the 5' end cDNA sequence shares

up to 90% sequence identity with known heat-shock 20kD like-protein P20 which includes human (XP_059039) and rat sequences (Inaguma et al. 1996: Accession No. P97541). HEAT-SHOCK 20 KD LIKE-PROTEIN P20 (belongs to the small heat shock protein (HSP20) family) which is related to stress

5 proteins.

The most closely relate gene family to vertebrate heat-shock 20kD like-protein P20 is the crystallin proteins which are approximately 46% identical the polypeptide sequence encoded by DACC-9.

10

DACC-10

The polypeptide sequence encoded by this cDNA sequence shares up to 95% sequence identity with known alpha 2 type V collagen preproproteins which includes human (Myers et al. 1985: Accession No. NP_000384) and
15 mouse sequences (Andrikopoulos et al. 1992: Accession No. NP_031763). Collagen alpha 2 type V is a subunit of type V collagen trimers. It is a minor connective tissue component which binds to DNA, Heparan sulphate, thrombospondin, heparin, and insulin. It is suggested to play an important role in collagen fibrillogenesis.

20 The most closely relate gene family to vertebrate alpha 2 V type collagen preproproteins is alpha 1 type II collagen which is approximately 62% identical the polypeptide sequence encoded by DACC-10.

DACC-11

25 The polypeptide sequence encoded by this cDNA sequence shares up to 97% sequence identity with known pro alpha 1(I) collagen which includes human (Chu et al. 1985: Accession No. AAB94054) and mouse sequences (Li et al. 1995: Accession No. P11087). Collagen alpha 1 type I is a subunit of type I collagen. It forms the fibrils of skin, tendon, ligaments and bones,
30 giving strength to connective tissues.

The most closely relate gene family to vertebrate pro alpha 1(I) collagen is alpha 1 type II collagen which is approximately 70% identical the polypeptide sequence encoded by DACC-11.

35 *Expression of DACC-7*

One aspect of the present invention provides a method of identifying and/or characterising the developmental position of mesenchymal cells, particularly during embryogenesis, the method comprising exposing a test sample including mesenchymal cell mRNA to a suitably-labelled nucleic acid probe with specifically hybridizes to a polynucleotide of the present invention and detecting hybridisation of said probe to said mRNA. Preferably, the test sample is a suitably prepared histological section.

One example of a method according to this aspect comprises the use of a 1.5 kb RNA probe prepared from clone DACC-7 according to standard techniques to identify chondrocytes and notochordal cells in active states of growth and differentiation. Figures 7 - 9 show histological sections of 12 - 14-week-old human foetal knee joints and spines subjected to *in-situ* hybridisation using the DACC-7 derived RNA probe illustrating strong expression by chondrocytes in growing cartilage. Similar studies with the DACC-7 probe using histological sections of human foetal spinal columns have demonstrated that notochordal cells and chondrocytes in the nucleus pulposus of the foetal disc also strongly express the gene product but fibrochondrocytes of the disc annulus fibrosis were less active (Figures 10 and 11). These observation was complemented by *in-situ* hybridisation using the same histological sections but a type II collagen RNA probe where uniform staining of chondrocytes and weaker staining for fibrochondrocytes of the annulus fibrosis was noted (Figures 10 and 11). A comparison of the intensity of cellular staining of histological sections made from joints of 12- (Figure 9) and 14-week-old (Figure 12) human fetuses with the DACC-7 derived RNA probe suggested that the expression of this gene product was similar in both age groups. In addition to the chondrocytes of growing foetal cartilage it was also demonstrated that the DACC-7 riboprobe was able to identify chondrocytes located in fibrillated cartilage from human osteoarthritic joints which were involved in attempted repair and regeneration of the extracellular matrix. These cells exhibited enhanced expression of DACC-7 as well as type II collagen, the chondrocyte phenotype protein, as illustrated by the sections shown in Figure 13. In contrast, it was found that the resting chondrocytes present in normal young ovine cartilages of the medial and lateral tibial plateaux failed to exhibit staining for the presence of DACC-7 expression confirming that DACC-7 expression is a

marker for cell undergoing proliferation during the active phases of cartilage growth and repair.

As would be expected from the origin of the gene probe, chondrocytes and particularly hypertrophic chondrocytes in the cartilaginous region of the growing deer antler also showed strong expression of DACC-7 and type II collagen gene expression by these same cells (Figure 14).

Growth Promoting Factors in Conditioned Media obtained from Deer Antler Cartilage Cells

Histological examination of the tissue sections obtained from DAC regions A, B, C (Figure 3) showed negligible staining for PGs in region A using Toluidine Blue but strong staining for PGs in sections from zones B and C (Figure 4). Blood vessels were present in each zone but the cell morphology evident in sections from zones B and C were of typical chondrocytes, zone A cells appeared more fibroblastic in appearance corresponding to pre-chondroblasts as described by Frasier *et al.* (1975). Sections of DAC from the proximate end of C zone showed the presence of hypertrophic chondrocytes accompanied by early mineralisation and increased vascular invasion (Figure 4).

DAC cells in alginate beads exhibited high incorporation of ^{35}S into PGs. Zone B, a region which is composed of mature chondrocyte-like cells and abundant cartilage matrix, showed statistically higher rates of PG synthesis than cells from zones A and C ($p < 0.05$) (Figure 16). Over the 72 h incubation period negligible amounts of ^{35}S -PGs were released into the media (Figure 16) confirming that minimal proteolytic modification of PGs were occurring in this culture system. Furthermore, studies of the mRNA obtained from these DAC cells using Northern blot analysis and a human aggrecan cRNA riboprobe confirmed that DAC cells maintained their phenotypic expression during these experiments (data not shown).

Cells from DAC zone B were also shown by their incorporation of ^3H -thymidine into DNA to be the more proliferative than cells from the other two zones ($p < 0.05$) (Figure 17) when they were cultured in the presence of 10% foetal bovine serum (FBS). Conditioned media (CM) collected from the alginate bead cultures of DAC cells when added to cultures of ovine articular chondrocytes in the absence of FBS induced a small stimulation of mitosis (Figure 18). However, it was noteworthy that CM from cells from zone A, the

prechondroblast zone, was more potent than from zone B ($p < 0.05$) (Figure 18). Under the same conditions DNA synthesis in SAC cultured in the presence of 10% FBS was increased by 30 - 35% (Figure 18).

It was found that foetal bovine serum could act synergistically with the growth factors produced by DAC cells since it augmented cell mitosis and synthesis of PGs. This was illustrated by the data shown in Figure 19 where it can be seen that replacing the FBS with FBS supplemented with 30% or 100% DAC-CM substantially increased ^{35}S -PG synthesis by ovine femoral chondrocytes. The amounts of PGs synthesised by using 100% DAC-CM which also contained 10% FBS was almost double that produced by 10% FBS alone (Figure 19). Condition media collected from cultures of regions A and B were shown to be more effective than from region C (Figure 19). Although similar profiles were obtained for cultures of chondrocytes obtained from the ovine tibial plateau (Figure 20), the cells from this joint region were found to be less responsive to DAC-CM than the femoral chondrocytes (Figure 20).

The ability of DAC-CM to stimulate ^{35}S -PG synthesis by ovine chondrocytes in the presence of FBS was also reflected in enhanced mitotic activity. As shown in Figure 21, DNA synthesis was more than doubled when either femoral or tibial SAC were cultured with 100% DAC-CM. Again cells from zones A and B produced higher amounts of growth factors than from DAC zone C ($p < 0.05$) and femoral sheep chondrocytes were more responsive to these factors than tibial chondrocytes.

The enhanced metabolic activity of SAC in the presence of DAC-CM was also reflected in increased mitochondrial activity using the MTT assay (Figure 22).

In all the previous experiments the conditioned media used was collected from the DAC cells maintained in culture for 24 h. In order to determine how long the growth factor(s) were elaborated by DAC cells CM was collected 1, 3, 5 and 7 days post-monolayer culture initiation. As is evident from Figure 23 the stimulatory effect of CM on ^{35}S -PG synthesis was more pronounced when collected from DAC cultures in the first 1 - 2 days irrespective of their origin.

A similar outcome was obtained using rabbit ear cartilage explant cultures as the target tissue but, as would be expected, far less ^{35}S -PGs were released into the media than in monolayer culture, the majority of ^{35}S -PGs being retained in the matrix (Figure 24).

The selective effects of the stimulatory factors produced by DAC cells in culture on chondrocytes were illustrated in the experiments using fibroblasts as target cells.

As is evident from Figure 25 addition of CM from DAC alginate bead
5 cultures or DAC monolayer cultures to 3T3 fibroblast cultures failed to stimulate but instead suppressed mitosis (relative to effects of FBS alone) as determined by the decreased incorporation of ³H-thymidine into DNA by these cells.

The present studies have shown that DAC cells can release soluble
10 factor(s) into culture media which can stimulate both DNA and PG synthesis by chondrocytes in monolayer or explant culture. This stimulatory effect was greatly enhanced when the media containing these factor(s) was supplemented with FBS which is known to contain a complex cocktail of growth factors, such as IGFs, basic and acidic FGFs, TGF- β , as well as
15 proteinase inhibitors and hormones.

The selectivity of the DAC derived factor(s) for chondrocytes and the amplification of its stimulatory effects in the presence of FBS suggests that their physiological role in the growing antler tip may be to direct and augment the multitude of blood borne growth factors which diffusing into the
20 tissues during the very active growth period.

Two-Dimensional gel electrophoresis sample images were obtained in triplicate for each of the 3 samples, for the pH gradients 3-6, 5-8 and 6-11. The 3 samples were derived from F4 - 24 h, F4 - 168 h and serum-free culture supernatants. Each image was cropped and grouped together as a triplicate
25 set of images. The 3 gels in each set were used to create a raw master reference gel that acted as a composite. This composite image was then used for comparative purposes in identifying protein spot differences between culture conditions. Regions of interest were then selected from the composite images that demonstrated differential display between F4 24 h and F4 168 h
30 culture supernatant samples. The differential display regions highlighted for each pH range showed that gels with 5-8 provided the best separation of proteins from the deer antler chondrocyte culture supernatant samples studied using Two-Dimensional Electrophoresis. Using this system changes in protein expression profile were observed between F4 24 h and F4 168 h
35 culture supernatant samples, indicating that protein expression differed over the time course studied. Regions exhibiting differential display were

selected, with differences in protein expression highlighted. A number of proteins present in the 24 h sample but absent in the 168 h sample are evident and were annotated. All were present at low levels but subjected to MS analysis.

5 Positive identification was only achieved for the proteins circled in Figure 26. MS analysis of the tryptic digests of these proteins revealed the Peptide A N-terminal amino acid sequence of FVEGL/IYQ/KVEL/IDTK (SEQ ID NO: 41) and Peptide B N-terminal amino acid sequence of EGL/IYQ/KV (SEQ ID NO: 42). By this method, leucine and isoleucine (L/I) are not
10 distinguishable, nor are glutamine and lysine (Q/K), differing in mass by only 0.04 Da. From the protein databases available both these proteins were identified as the protein transthyretin.

Transthyretin is a thyroid hormone-binding protein which forms tight protein-protein complex with the retinol-binding protein (RBP). The
15 formation of the complex with RBP stabilises the binding of retinol to RBP. The term refers to the fact that it is a transport protein for both thyroxine and retinol (vitamin A). Transthyretin is also one of the precursor proteins commonly found in amyloid deposits (transthyretin-associated amyloidosis disease).

20 The finding that one of the proteins expressed by the-DAC derived factor(s) during the early stages but not latter stages of culture was transthyretin was consistent with the observed stimulatory effect of these supernatants. The effect probably being mediated by the ability of transthyretin to carry thyroxine in complex with retinol into the cell and thus
25 promote the proliferation of cartilage and its subsequent conversion the bone. This is the first report of the production of transthyretin by chondrocytes but is consistent with the known role of this protein in the growth and development of other mesenchymal tissues (Sakabe et al. 1999; Barron et al. 1998; Hamazaki et al. 2001).

30 The present inventors have shown that the expression of the mRNA for the type II procollagen and proteoglycans can be upregulated in cultures of human and ovine chondrocytes by genes derived from deer antler chondrocytes. This response can be modified by concomitant exposure of these cells to a variety of hormones and endocrine growth factors including:
35 insulin-like growth factor (IGF-1), TGF-beta, FGFs, VEGFs, morphogenic bone factors, thyroid hormones (thyroxine), parathyroid hormone related protein

(PTHrP), sex hormones, luteinizing hormone (LH) and prolactin and even conditioned medium obtained by culturing the deer antler chondrocytes themselves. One or a combination of these hormones and/or growth factors may be used to increase the rate of proliferation and thus number of DACC
5 gene transfected chondrocytes obtained from the original biopsy thereby providing sufficient numbers of cells for implantation into connective tissue defects or to stored cryogenically for transplantation at a later date. One of the proteins identified in the supernatants obtained from the deer antler cell cultures which produce these stimulatory activity to chondrocytes was
10 transthyretin, a thyroid hormone-binding protein and forms complexes with retinol-binding protein, known to be involved in embryonic development (Sakabe et al. 1999; Barron et al. 1998; Ingenbleek and Bernstein, 1999; Stark et al. 2001; Hamazaki et al. 2001; Varga and Vajtai, 1998).

The present results identify a method of improving mesenchymal cell
15 growth, repair, regeneration or restoration of cartilage, tendon, meniscal and disc defects which would restore their function and decrease the rate of development of OA in the joint. This procedure would require either surgically obtaining a small biopsy of cartilage adjacent to the defect, or from within the target disc, isolating the chondrocytes from these biopsies,
20 establishing them in culture and transfecting them with a gene(s) which the present inventors have identified in the rapidly growing cartilage cells of deer antler and replacing the transfected chondrocytes back into the defect using a suitable carrier, or artificial matrix, to maintain them in place. Another procedure would require transfecting cartilage adjacent to the defect, or from
25 within the target disc, *in vivo* as described previously and in detail by Goomer *et al.* (2000). These modified chondrocytes in response to the normal mechanical and nutritional factors acting on the disc and cartilage plug *in vivo* would stimulate the transformed cells to proliferate and synthesise a new matrix capable of repairing the defect.

30

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to
35 be considered in all respects as illustrative and not restrictive.

All publications discussed above are incorporated herein in their entirety.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

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CLAIMS:

1. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
 - 5 a) a sequence as shown in SEQ ID NO:1,
 - b) a sequence as shown in SEQ ID NO:2,
 - c) a sequence as shown in SEQ ID NO:3, and
 - d) a sequence which is at least 50% identical to any one of (a) to (c).
- 10 2. The method of claim 1, wherein the polypeptide is at least 80% identical to any one of (a) to (c).
3. The method of claim 1, wherein the polypeptide is at least 90% identical to any one of (a) to (c).
- 15 4. The method of claim 1, wherein the polypeptide is at least 95% identical to any one of (a) to (c).
5. A method of stimulating cell growth and/or division, the method
20 comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
 - a) a sequence as shown in SEQ ID NO:4,
 - b) a sequence as shown in SEQ ID NO:5,
 - c) a sequence as shown in SEQ ID NO:6, and
 - 25 d) a sequence which is at least 70% identical to any one of (a) to (c).
6. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
 - 30 a) a sequence as shown in SEQ ID NO:7,
 - b) a sequence as shown in SEQ ID NO:8,
 - c) a sequence as shown in SEQ ID NO:9, and
 - d) a sequence which is at least 80% identical to any one of (a) to (c).

7. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:10,
 - 5 b) a sequence as shown in SEQ ID NO:11,
 - c) a sequence as shown in SEQ ID NO:12, and
 - d) a sequence which is at least 85% identical to any one of (a) to (c).
8. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:13,
 - b) a sequence as shown in SEQ ID NO:14, and
 - c) a sequence which is at least 70% identical to any one of (a) or (b).
- 15 9. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:15,
 - 20 b) a sequence as shown in SEQ ID NO:16, and
 - c) a sequence which is at least 50% identical to any one of (a) or (b).
10. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- 25 a) a sequence as shown in SEQ ID NO:17, and
 - b) a sequence which is at least 60% identical to a).
11. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:18,
 - b) a sequence as shown in SEQ ID NO:19,
 - c) a sequence as shown in SEQ ID NO:20, and
 - 35 d) a sequence which is at least 50% identical to any one of (a) to (c).

12. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:21,
 - 5 b) a sequence as shown in SEQ ID NO:22,
 - c) a sequence as shown in SEQ ID NO:23, and
 - d) a sequence which is at least 65% identical to any one of (a) to (c).
13. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:24,
 - b) a sequence as shown in SEQ ID NO:25,
 - c) a sequence as shown in SEQ ID NO:26, and
 - 15 d) a sequence which is at least 75% identical to any one of (a) to (c).
14. A method of stimulating cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a polypeptide comprising a sequence selected from the group consisting of:
- 20 a) a sequence as shown in SEQ ID NO:27, and
 - b) a sequence which is at least 35% identical to a).
15. The method according to any one of the preceding claims, wherein the cell is a somatic cell.
- 25 16. The method according to claim 15, wherein the somatic cell is a mesenchymal cell.
17. The method according to claim 16, wherein the mesenchymal cell is selected from the group consisting of: chondrocytes and osteocytes.
- 30 18. The method according to any one of the preceding claims, wherein the polypeptide is provided by introducing into the cell an expression vector encoding the polypeptide.

19. The method according to any one of the preceding claims, wherein the cell is removed from an animal, cultured *in vitro*, transformed or transfected with a polynucleotide encoding the polypeptide and then placed back into an animal.
- 5 20. The method according to any one of claims 1 to 18, wherein the cell is transformed or transfected *in vivo* with a polynucleotide encoding the polypeptide.
- 10 21. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 15 a) a sequence as shown in SEQ ID NO:1,
b) a sequence as shown in SEQ ID NO:2,
c) a sequence as shown in SEQ ID NO:3, and
d) a sequence which is at least 50% identical to any one of (a) to (c).
22. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 20 a) a sequence as shown in SEQ ID NO:4,
b) a sequence as shown in SEQ ID NO:5,
25 c) a sequence as shown in SEQ ID NO:6, and
d) a sequence which is at least 70% identical to any one of (a) to (c).
23. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 30 a) a sequence as shown in SEQ ID NO:7,
b) a sequence as shown in SEQ ID NO:8,
c) a sequence as shown in SEQ ID NO:9, and
35 d) a sequence which is at least 80% identical to any one of (a) to (c):

24. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 5 a) a sequence as shown in SEQ ID NO:10,
 - b) a sequence as shown in SEQ ID NO:11,
 - c) a sequence as shown in SEQ ID NO:12, and
 - d) a sequence which is at least 85% identical to any one of (a) to (c).
- 10 25. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 15 a) a sequence as shown in SEQ ID NO:13,
 - b) a sequence as shown in SEQ ID NO:14, and
 - c) a sequence which is at least 70% identical to any one of (a) or (b).
26. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which
- 20 hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:15,
 - b) a sequence as shown in SEQ ID NO:16, and
 - c) a sequence which is at least 50% identical to any one of (a) or (b).
- 25 27. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:
- 30 a) a sequence as shown in SEQ ID NO:17, and
 - b) a sequence which is at least 60% identical to a).
28. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which
- 35 hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:18,
- b) a sequence as shown in SEQ ID NO:19,
- c) a sequence as shown in SEQ ID NO:20, and
- d) a sequence which is at least 50% identical to any one of (a) to (c).

5

29. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:

10

- a) a sequence as shown in SEQ ID NO:21,
- b) a sequence as shown in SEQ ID NO:22,
- c) a sequence as shown in SEQ ID NO:23, and
- d) a sequence which is at least 65% identical to any one of (a) to (c).

15

30. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:

20

- a) a sequence as shown in SEQ ID NO:24,
- b) a sequence as shown in SEQ ID NO:25,
- c) a sequence as shown in SEQ ID NO:26, and
- d) a sequence which is at least 75% identical to any one of (a) to (c).

25

31. A method of inhibiting cell growth and/or division, the method comprising contacting, or inserting into, an animal cell a compound which hybridizes to, and inhibits the translation of, a polynucleotide encoding a polypeptide selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:27, and
- b) a sequence which is at least 35% identical to a).

30

32. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

35

- i) exposing the polypeptide to a candidate agent, and
- ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,

wherein the polypeptide has a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:1,
- b) a sequence as shown in SEQ ID NO:2,
- 5 c) a sequence as shown in SEQ ID NO:3, and
- d) a sequence which is at least 50% identical to any one of (a) to (c).

33. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method
10 comprising

- i) exposing the polypeptide to a candidate agent, and
 - ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
- wherein the polypeptide has a sequence selected from the group consisting
15 of:

- a) a sequence as shown in SEQ ID NO:4,
- b) a sequence as shown in SEQ ID NO:5,
- c) a sequence as shown in SEQ ID NO:6, and
- 20 d) a sequence which is at least 70% identical to any one of (a) to (c).

34. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- i) exposing the polypeptide to a candidate agent, and
 - 25 ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
- wherein the polypeptide has a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:7,
- 30 b) a sequence as shown in SEQ ID NO:8,
- c) a sequence as shown in SEQ ID NO:9, and
- d) a sequence which is at least 80% identical to any one of (a) to (c).

35. A method of identifying an agent that modulates the activity of a
35 polypeptide that stimulates animal cell growth and/or division, the method comprising

i) exposing the polypeptide to a candidate agent, and
ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
wherein the polypeptide has a sequence selected from the group consisting
5 of:

- a) a sequence as shown in SEQ ID NO:10,
- b) a sequence as shown in SEQ ID NO:11,
- c) a sequence as shown in SEQ ID NO:12, and
- d) a sequence which is at least 85% identical to any one of (a) to (c).

10

36. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- 15 i) exposing the polypeptide to a candidate agent, and
ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
wherein the polypeptide has a sequence selected from the group consisting of:

- 20 a) a sequence as shown in SEQ ID NO:13,
b) a sequence as shown in SEQ ID NO:14, and
c) a sequence which is at least 70% identical to any one of (a) or (b).

37. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method
25 comprising

- i) exposing the polypeptide to a candidate agent, and
ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
wherein the polypeptide has a sequence selected from the group consisting
30 of:

- a) a sequence as shown in SEQ ID NO:15,
- b) a sequence as shown in SEQ ID NO:16, and
- c) a sequence which is at least 50% identical to any one of (a) or (b).

38. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- i) exposing the polypeptide to a candidate agent, and
- 5 ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division, wherein the polypeptide has a sequence selected from the group consisting of:
 - a) a sequence as shown in SEQ ID NO:17, and
 - 10 b) a sequence which is at least 60% identical to a).

39. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- 15 i) exposing the polypeptide to a candidate agent, and
- ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division, wherein the polypeptide has a sequence selected from the group consisting of:
 - 20 a) a sequence as shown in SEQ ID NO:18,
 - b) a sequence as shown in SEQ ID NO:19,
 - c) a sequence as shown in SEQ ID NO:20, and
 - d) a sequence which is at least 50% identical to any one of (a) to (c).

25 40. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising

- i) exposing the polypeptide to a candidate agent, and
- ii) assessing the ability of the candidate agent to modulate the ability of
- 30 the polypeptide to stimulate cell growth and/or division, wherein the polypeptide has a sequence selected from the group consisting of:
 - a) a sequence as shown in SEQ ID NO:21,
 - b) a sequence as shown in SEQ ID NO:22,
 - 35 c) a sequence as shown in SEQ ID NO:23, and
 - d) a sequence which is at least 65% identical to any one of (a) to (c).

41. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising
- 5 i) exposing the polypeptide to a candidate agent, and
 ii) assessing the ability of the candidate agent to modulate the ability of the polypeptide to stimulate cell growth and/or division,
wherein the polypeptide has a sequence selected from the group consisting of:
- 10 a) a sequence as shown in SEQ ID NO:24,
 b) a sequence as shown in SEQ ID NO:25,
 c) a sequence as shown in SEQ ID NO:26, and
 d) a sequence which is at least 75% identical to any one of (a) to (c).
- 15 42. A method of identifying an agent that modulates the activity of a polypeptide that stimulates animal cell growth and/or division, the method comprising
- i) exposing the polypeptide to a candidate agent, and
 ii) assessing the ability of the candidate agent to modulate the ability of
- 20 the polypeptide to stimulate cell growth and/or division,
wherein the polypeptide has a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:27, and
 b) a sequence which is at least 35% identical to a).
- 25 43. The method according to any one of claims 32 to 42, wherein the agent inhibits the ability of the polypeptide to stimulate cell growth and/or division.
44. The method according to any one of claims 32 to 42, wherein the agent
- 30 enhances the ability of the polypeptide to stimulate cell growth and/or division.
45. The method according to any one of the preceding claims, wherein the animal cell is a mammalian cell.

46. A method of stimulating mesenchymal cell growth and/or division, the method comprising exposing animal mesenchymal cells to conditioned media, or an active fraction thereof, obtained from deer antler cartilage cells.

5 47. The method according to claim 46, wherein the deer antler cartilage cells are selected from the group consisting of: prechondrocytes, mature chondrocytes, hypertrophic chondrocytes, or a combination thereof.

48. The method according to claim 46 or claim 47, wherein the method
10 further comprises exposing the cells to a growth factor.

49. The method according to claim 48, wherein the growth factor is selected from the group consisting of: insulin-like growth factor (IGF-1), TGF-beta, fibroblast growth factor (FGF), vascular endothelial growth factor
15 (VEGF), morphogenic bone factors, thyroid hormones (thyroxine), parathyroid hormone related protein (PTHrP), sex hormones, luteinizing hormone (LH) and prolactin.

50. A substantially purified polypeptide comprising a sequence selected
20 from the group consisting of:

- a) a sequence as shown in SEQ ID NO:1, and
- b) a sequence which is at least 91% identical to a),

wherein the polypeptide is capable of stimulating animal cell growth and/or division.

25

51. The polypeptide according to claim 50, wherein the sequence is least 95% identical to a).

52. The polypeptide according to claim 50, wherein the sequence is least
30 99% identical to a).

53. A substantially purified polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:4, and
- 35 b) a sequence which is at least 99% identical to a),

wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or a structural component of extracellular matrix.

5 54. A substantially purified polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:7, and
- b) a sequence which is at least 99% identical to a),

10 wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or subunit involved in protein synthesis.

55. A substantially purified polypeptide comprising a sequence selected from the group consisting of:

- 15 a) a sequence as shown in SEQ ID NO:13, and
- b) a sequence which is at least 90% identical to a),

wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or altering chromatin structure.

20

56. A substantially purified polypeptide comprising a sequence selected from the group consisting of:

- a) a sequence as shown in SEQ ID NO:15, and
- b) a sequence which is at least 99% identical to a),

25 wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or regulating cell migration.

57. A substantially purified polypeptide comprising a sequence selected from the group consisting of:

- 30 a) a sequence as shown in SEQ ID NO:18, and
- b) a sequence which is at least 91% identical to a),

wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or responses to
35 cell stress.

58. A substantially purified polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:21, and
 - b) a sequence which is at least 96% identical to a),
- 5 wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or a component of connective tissue, or collagen fibrillogenesis.
59. A substantially purified polypeptide comprising a sequence selected from the group consisting of:
- a) a sequence as shown in SEQ ID NO:24, and
 - b) a sequence which is at least 98% identical to a),
- 10 wherein the polypeptide has a biological activity selected from the group consisting of: stimulating animal cell growth and/or division, or a component of collagen.
- 15 of collagen.
60. A fusion protein comprising a polypeptide according to any one of claims 50 to 59.
- 20 61. An isolated polynucleotide encoding a polypeptide according to any one of claims 50 to 60.
62. The polynucleotide according to claim 61 comprising a sequence according to any one of SEQ ID NO:28, 29, 31 to 33, or 35 to 38.
- 25 63. An isolated polynucleotide comprising a sequence provided as SEQ ID NO:30.
64. An isolated polynucleotide comprising a sequence provided as SEQ ID NO:34.
- 30 65. An antisense polynucleotide which hybridizes under high stringency conditions to a polynucleotide according to any one of claims 61 to 64.
- 35 66. A vector comprising the polynucleotide according to any one of claims 61 to 65.

67. The vector according to claim 66, wherein the polynucleotide is operably linked to a promoter.
- 5 68. A host cell transfected or transformed with a vector according to claim 66 or claim 67.
69. The host cell of claim 68 which is a mammalian cell.
- 10 70. A method according to any one of claims 14, 31, 42 and 46 wherein the increased cell division and/or matrix gene expression by chondrocytes results from the action of transthyretin.

SEQUENCE LISTING

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The University of Sydney

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11/75

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12/75

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Pro Val Cys Phe Leu
 1415

<210> 6
 <211> 1487
 <212> PRT
 <213> Mus musculus

<400> 6

Met Ile Arg Leu Gly Ala Pro Gln Ser Leu Val Leu Leu Thr Leu Leu
 1 5 10 15

Ile Ala Ala Val Leu Arg Cys Gln Gly Gln Asp Ala Gln Glu Ala Gly
 20 25 30

Ser Cys Leu Gln Asn Gly Gln Arg Tyr Lys Asp Lys Asp Val Trp Lys
 35 40 45

Pro Ser Ser Cys Arg Ile Cys Val Cys Asp Thr Gly Asn Val Leu Cys
 50 55 60

Asp Asp Ile Ile Cys Glu Asp Pro Asp Cys Leu Asn Pro Glu Ile Pro
 65 70 75 80

Phe Gly Glu Cys Cys Pro Ile Cys Pro Ala Asp Leu Ala Thr Ala Ser
 85 90 95

Gly Lys Leu Gly Pro Lys Gly Gln Lys Gly Glu Pro Gly Asp Ile Arg
 100 105 110

Asp Ile Ile Gly Pro Arg Gly Pro Pro Gly Pro Gln Gly Pro Ala Gly
 115 120 125
 Glu Gln Gly Pro Arg Gly Asp Arg Gly Asp Lys Gly Glu Lys Gly Ala
 130 135 140
 Pro Gly Pro Arg Gly Arg Asp Gly Glu Pro Gly Thr Pro Gly Asn Pro
 145 150 155 160
 Gly Pro Ala Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Leu Ser Ala
 165 170 175
 Gly Asn Phe Ala Ala Gln Met Ala Gly Gly Tyr Asp Glu Lys Ala Gly
 180 185 190
 Gly Ala Gln Met Gly Val Met Gln Gly Pro Met Gly Pro Met Gly Pro
 195 200 205
 Arg Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro Gln Gly Phe Gln
 210 215 220
 Gly Asn Pro Gly Glu Pro Gly Glu Pro Gly Val Ser Gly Pro Met Gly
 225 230 235 240
 Pro Arg Gly Pro Pro Gly Pro Ala Gly Lys Pro Gly Asp Asp Gly Glu
 245 250 255
 Ala Gly Lys Pro Gly Lys Ser Gly Glu Arg Gly Leu Pro Gly Pro Gln
 260 265 270
 Gly Ala Arg Gly Phe Pro Gly Thr Pro Gly Leu Pro Gly Val Lys Gly
 275 280 285
 His Arg Gly Tyr Pro Gly Leu Asp Gly Ala Lys Gly Glu Ala Gly Ala
 290 295 300
 Pro Gly Val Lys Gly Glu Ser Gly Ser Pro Gly Glu Asn Gly Ser Pro
 305 310 315 320
 Gly Pro Met Gly Pro Arg Gly Leu Pro Gly Glu Arg Gly Arg Thr Gly
 325 330 335
 Pro Ala Gly Ala Ala Gly Ala Arg Gly Asn Asp Gly Gln Pro Gly Pro
 340 345 350

Ala Gly Pro Pro Gly Pro Val Gly Pro Ala Gly Gly Pro Gly Phe Pro
 355 360 365

Gly Ala Pro Gly Ala Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly
 370 375 380

Pro Glu Gly Ala Gln Gly Ser Arg Gly Glu Pro Gly Asn Pro Gly Ser
 385 390 395 400

Pro Gly Pro Ala Gly Ala Ser Gly Asn Pro Gly Thr Asp Gly Ile Pro
 405 410 415

Gly Ala Lys Gly Ser Ala Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly
 420 425 430

Phe Pro Gly Pro Arg Gly Pro Pro Gly Pro Gln Gly Ala Thr Gly Pro
 435 440 445

Leu Gly Pro Lys Gly Gln Ala Gly Glu Pro Gly Ile Ala Gly Phe Lys
 450 455 460

Gly Asp Gln Gly Pro Lys Gly Glu Thr Gly Pro Ala Gly Pro Gln Gly
 465 470 475 480

Ala Pro Gly Pro Ala Gly Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu
 485 490 495

Pro Gly Gly Ala Gly Pro Ile Gly Pro Pro Gly Glu Arg Gly Ala Pro
 500 505 510

Gly Asn Arg Gly Phe Pro Gly Gln Asp Gly Leu Ala Gly Pro Lys Gly
 515 520 525

Ala Pro Gly Glu Arg Gly Pro Ser Gly Leu Ala Gly Pro Lys Gly Ala
 530 535 540

Asn Gly Asp Pro Gly Arg Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg
 545 550 555 560

Gly Leu Thr Gly Arg Pro Gly Asp Ala Gly Pro Gln Gly Lys Val Gly
 565 570 575

Pro Ser Gly Ala Pro Gly Glu Asp Gly Arg Pro Gly Pro Pro Gly Pro
 580 585 590

15/75

Gln Gly Ala Arg Gly Gln Pro Gly Val Met Gly Phe Pro Gly Pro Lys
 595 600 605

Gly Ala Asn Gly Glu Pro Gly Lys Ala Gly Glu Lys Gly Leu Ala Gly
 610 615 620

Ala Pro Gly Leu Arg Gly Leu Pro Gly Lys Asp Gly Glu Thr Gly Ala
 625 630 635 640

Ala Gly Pro Pro Gly Pro Ser Gly Pro Ala Gly Glu Arg Gly Glu Gln
 645 650 655

Gly Ala Pro Gly Pro Ser Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly
 660 665 670

Pro Pro Gly Glu Gly Gly Lys Gln Gly Asp Gln Gly Ile Pro Gly Glu
 675 680 685

Ala Gly Ala Pro Gly Leu Val Gly Pro Arg Gly Glu Arg Gly Phe Pro
 690 695 700

Gly Glu Arg Gly Ser Pro Gly Ala Gln Gly Leu Gln Gly Pro Arg Gly
 705 710 715 720

Leu Pro Gly Thr Pro Gly Thr Asp Gly Pro Lys Gly Ala Ala Gly Pro
 725 730 735

Asp Gly Pro Pro Gly Ala Gln Gly Pro Pro Gly Leu Gln Gly Met Pro
 740 745 750

Gly Glu Arg Gly Ala Ala Gly Ile Ala Gly Pro Lys Gly Asp Arg Gly
 755 760 765

Asp Val Gly Glu Lys Gly Pro Glu Gly Ala Pro Gly Lys Asp Gly Gly
 770 775 780

Arg Gly Leu Thr Gly Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Asn
 785 790 795 800

Gly Glu Lys Gly Glu Val Gly Pro Pro Gly Pro Ser Gly Ser Thr Gly
 805 810 815

Ala Arg Gly Ala Pro Gly Glu Pro Gly Glu Thr Gly Pro Pro Gly Pro
 820 825 830

Ala Gly Phe Ala Gly Pro Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys
835 840 845

Gly Asp Gln Gly Glu Ala Gly Gln Lys Gly Asp Ala Gly Ala Pro Gly
850 855 860

Pro Gln Gly Pro Ser Gly Ala Pro Gly Pro Gln Gly Pro Thr Gly Val.
865 870 875 880

Thr Gly Pro Lys Gly Ala Arg Gly Ala Gln Gly Pro Pro Gly Ala Thr
885 890 895

Gly Phe Pro Gly Ala Ala Gly Arg Val Gly Pro Pro Gly Ala Asn Gly
900 905 910

Asn Pro Gly Pro Ala Gly Pro Pro Gly Pro Ala Gly Lys Asp Gly Pro
915 920 925

Lys Gly Val Arg Gly Asp Ser Gly Pro Pro Gly Arg Ala Gly Asp Pro
930 935 940

Gly Leu Gln Gly Pro Ala Gly Ala Pro Gly Glu Lys Gly Glu Pro Gly
945 950 955 960

Asp Asp Gly Pro Ser Gly Leu Asp Gly Pro Pro Gly Pro Gln Gly Leu
965 970 975

Ala Gly Gln Arg Gly Ile Val Gly Leu Pro Gly Gln Arg Gly Glu Arg
980 985 990

Gly Phe Pro Gly Leu Pro Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly
995 1000 1005

Ala Pro Gly Ala Ser Gly Asp Arg Gly Pro Pro Gly Pro Val Gly
1010 1015 1020

Pro Pro Gly Leu Thr Gly Pro Ala Gly Glu Pro Gly Arg Glu Gly
1025 1030 1035

Ser Pro Gly Ala Asp Gly Pro Pro Gly Arg Asp Gly Ala Ala Gly
1040 1045 1050

Val Lys Gly Asp Arg Gly Glu Thr Gly Ala Leu Gly Ala Pro Gly
1055 1060 1065

Ala Pro	Gly Pro Pro Gly	Ser	Pro Gly Pro Ala Gly	Pro Thr Gly
1070		1075		1080
Lys Gln	Gly Asp Arg Gly	Glu	Ala Gly Ala Gln Gly	Pro Met Gly
1085		1090		1095
Pro Ser	Gly Pro Ala Gly	Ala	Arg Gly Ile Ala Gly	Pro Gln Gly
1100		1105		1110
Pro Arg	Gly Asp Lys Gly	Glu	Ser Gly Glu Gln Gly	Glu Arg Gly
1115		1120		1125
Leu Lys	Gly His Arg Gly	Phe	Thr Gly Leu Gln Gly	Leu Pro Gly
1130		1135		1140
Pro Pro	Gly Pro Ser Gly	Asp	Gln Gly Ala Ser Gly	Pro Ala Gly
1145		1150		1155
Pro Ser	Gly Pro Arg Gly	Pro	Pro Gly Pro Val Gly	Pro Ser Gly
1160		1165		1170
Lys Asp	Gly Ser Asn Gly	Ile	Pro Gly Pro Ile Gly	Pro Pro Gly
1175		1180		1185
Pro Arg	Gly Arg Ser Gly	Glu	Thr Gly Pro Val Gly	Pro Pro Gly
1190		1195		1200
Ser Pro	Gly Pro Pro Gly	Pro	Pro Gly Pro Pro Gly	Pro Gly Ile
1205		1210		1215
Asp Met	Ser Ala Phe Ala	Gly	Leu Gly Gln Arg Glu	Lys Gly Pro
1220		1225		1230
Asp Pro	Met Gln Tyr Met	Arg	Ala Asp Glu Ala Asp	Ser Thr Leu
1235		1240		1245
Arg Gln	His Asp Val Glu	Val	Asp Ala Thr Leu Lys	Ser Leu Asn
1250		1255		1260
Asn Gln	Ile Glu Ser Ile	Arg	Ser Pro Asp Gly Ser	Arg Lys Asn
1265		1270		1275
Pro Ala	Arg Thr Cys Gln	Asp	Leu Lys Leu Cys His	Pro Glu Trp
1280		1285		1290

18/75

Lys Ser Gly Asp Tyr Trp Ile Asp Pro Asn Gln Gly Cys Thr Leu
1295 1300 1305

Asp Ala Met Lys Val Phe Cys Asn Met Glu Thr Gly Glu Thr Cys
1310 1315 1320

Val Tyr Pro Asn Pro Ala Thr Val Pro Arg Lys Asn Trp Trp Ser
1325 1330 1335

Ser Lys Ser Lys Glu Lys Lys His Ile Trp Phe Gly Glu Thr Met
1340 1345 1350

Asn Gly Gly Phe His Phe Ser Tyr Gly Asp Gly Asn Leu Ala Pro
1355 1360 1365

Asn Thr Ala Asn Val Gln Met Thr Phe Leu Arg Leu Leu Ser Thr
1370 1375 1380

Glu Gly Ser Gln Asn Ile Thr Tyr His Cys Lys Asn Ser Ile Ala
1385 1390 1395

Tyr Leu Asp Glu Ala Ala Gly Asn Leu Lys Lys Ala Leu Leu Ile
1400 1405 1410

Gln Gly Ser Asn Asp Val Glu Met Arg Ala Glu Gly Asn Ser Arg
1415 1420 1425

Phe Thr Tyr Thr Ala Leu Lys Asp Gly Cys Thr Lys His Thr Gly
1430 1435 1440

Lys Trp Gly Lys Thr Val Ile Glu Tyr Arg Ser Gln Lys Thr Ser
1445 1450 1455

Arg Leu Pro Ile Ile Asp Ile Ala Pro Met Asp Ile Gly Gly Ala
1460 1465 1470

Glu Gln Glu Phe Gly Val Asp Ile Gly Pro Val Cys Phe Leu
1475 1480 1485

<210> 7

<211> 293

<212> PRT

<213> Cervus elaphus

<400> 7

Met Ala Asp Asp Ala Gly Ala Ala Gly Gly Pro Gly Gly Pro Gly Gly
1 5 10 15

19/75

Pro Gly Met Gly Gly Arg Gly Gly Phe Arg Gly Gly Phe Gly Ser Gly
 20 25 30

Val Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg
 35 40 45

Gly Ala Arg Gly Gly Lys Ala Glu Asp Lys Glu Trp Leu Pro Val Thr
 50 55 60

Lys Leu Gly Arg Leu Val Lys Asp Met Lys Ile Lys Ser Leu Glu Glu
 65 70 75 80

Ile Tyr Leu Phe Ser Leu Pro Ile Lys Glu Ser Glu Ile Ile Asp Phe
 85 90 95

Phe Leu Gly Ala Ser Leu Lys Asp Glu Val Leu Lys Ile Met Pro Val
 100 105 110

Gln Lys Gln Thr Arg Ala Gly Gln Arg Thr Arg Phe Lys Ala Phe Val
 115 120 125

Ala Ile Gly Asp Tyr Asn Gly His Val Gly Leu Gly Val Lys Cys Ser
 130 135 140

Lys Glu Val Ala Thr Ala Ile Arg Gly Ala Ile Ile Leu Ala Lys Leu
 145 150 155 160

Ser Ile Val Pro Val Arg Arg Gly Tyr Trp Gly Asn Lys Ile Gly Lys
 165 170 175

Pro His Thr Val Pro Cys Lys Val Thr Gly Arg Cys Gly Ser Val Leu
 180 185 190

Val Arg Leu Ile Pro Ala Pro Arg Gly Thr Gly Ile Val Ser Ala Pro
 195 200 205

Val Pro Lys Lys Leu Leu Met Met Ala Gly Ile Asp Asp Cys Tyr Thr
 210 215 220

Ser Ala Arg Gly Cys Thr Ala Thr Leu Gly Asn Phe Ala Lys Ala Thr
 225 230 235 240

Phe Asp Ala Ile Ser Lys Thr Tyr Ser Tyr Leu Thr Pro Asp Leu Trp
 245 250 255

20/75

Lys Glu Thr Val Phe Thr Lys Ser Pro Tyr Gln Glu Phe Thr Asp His
 260 265 270

Leu Val Lys Thr His Thr Arg Val Ser Val Gln Arg Thr Gln Ala Pro
 275 280 285

Ala Val Ala Thr Thr
 290

<210> 8
 <211> 293
 <212> PRT
 <213> Homo sapiens

<400> 8

Met Ala Asp Asp Ala Gly Ala Ala Gly Gly Pro Gly Gly Pro Gly Gly
 1 5 10 15

Pro Gly Met Gly Asn Arg Gly Gly Phe Arg Gly Gly Phe Gly Ser Gly
 20 25 30

Ile Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg
 35 40 45

Gly Ala Arg Gly Gly Lys Ala Glu Asp Lys Glu Trp Met Pro Val Thr
 50 55 60

Lys Leu Gly Arg Leu Val Lys Asp Met Lys Ile Lys Ser Leu Glu Glu
 65 70 75 80

Ile Tyr Leu Phe Ser Leu Pro Ile Lys Glu Ser Glu Ile Ile Asp Phe
 85 90 95

Phe Leu Gly Ala Ser Leu Lys Asp Glu Val Leu Lys Ile Met Pro Val
 100 105 110

Gln Lys Gln Thr Arg Ala Gly Gln Arg Thr Arg Phe Lys Ala Phe Val
 115 120 125

Ala Ile Gly Asp Tyr Asn Gly His Val Gly Leu Gly Val Lys Cys Ser
 130 135 140

Lys Glu Val Ala Thr Ala Ile Arg Gly Ala Ile Ile Leu Ala Lys Leu
 145 150 155 160

Ser Ile Val Pro Val Arg Arg Gly Tyr Trp Gly Asn Lys Ile Gly Lys
 165 170 175

21/75

Pro His Thr Val Pro Cys Lys Val Thr Gly Arg Cys Gly Ser Val Leu
180 185 190

Val Arg Leu Ile Pro Ala Pro Arg Gly Thr Gly Ile Val Ser Ala Pro
195 200 205

Val Pro Lys Lys Leu Leu Met Met Ala Gly Ile Asp Asp Cys Tyr Thr
210 215 220

Ser Ala Arg Gly Cys Thr Ala Thr Leu Gly Asn Phe Ala Lys Ala Thr
225 230 235 240

Phe Asp Ala Ile Ser Lys Thr Tyr Ser Tyr Leu Thr Pro Asp Leu Trp
245 250 255

Lys Glu Thr Val Phe Thr Lys Ser Pro Tyr Gln Glu Phe Thr Asp His
260 265 270

Leu Val Lys Thr His Thr Arg Val Ser Val Gln Arg Thr Gln Ala Pro
275 280 285

Ala Val Ala Thr Thr
290

<210> 9
<211> 293
<212> PRT
<213> Mus musculus

<400> 9

Met Ala Asp Asp Ala Gly Ala Ala Gly Gly Pro Gly Gly Pro Gly Gly
1 5 10 15

Pro Gly Leu Gly Gly Arg Gly Gly Phe Arg Gly Gly Phe Gly Ser Gly
20 25 30

Leu Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg Gly Arg
35 40 45

Gly Ala Arg Gly Gly Lys Ala Glu Asp Lys Glu Trp Ile Pro Val Thr
50 55 60

Lys Leu Gly Arg Leu Val Lys Asp Met Lys Ile Lys Ser Leu Glu Glu
65 70 75 80

Ile Tyr Leu Phe Ser Leu Pro Ile Lys Glu Ser Glu Ile Ile Asp Phe
85 90 95

Phe Leu Gly Ala Ser Leu Lys Asp Glu Val Leu Lys Ile Met Pro Val
 100 105 110

Gln Lys Gln Thr Arg Ala Gly Gln Arg Thr Arg Phe Lys Ala Phe Val
 115 120 125

Ala Ile Gly Asp Tyr Asn Gly His Val Gly Leu Gly Val Lys Cys Ser
 130 135 140

Lys Glu Val Ala Thr Ala Ile Arg Gly Ala Ile Ile Leu Ala Lys Leu
 145 150 155 160

Ser Ile Val Pro Val Arg Arg Gly Tyr Trp Gly Asn Lys Ile Gly Lys
 165 170 175

Pro His Thr Val Pro Cys Lys Val Thr Gly Arg Cys Gly Ser Val Leu
 180 185 190

Val Arg Leu Ile Pro Ala Pro Arg Gly Thr Gly Ile Val Ser Ala Pro
 195 200 205

Val Pro Lys Lys Leu Leu Met Met Ala Gly Ile Asp Asp Cys Tyr Thr
 210 215 220

Ser Ala Arg Gly Cys Thr Ala Thr Leu Gly Asn Phe Ala Lys Ala Thr
 225 230 235 240

Phe Asp Ala Ile Ser Lys Thr Tyr Ser Tyr Leu Thr Pro Asp Leu Trp
 245 250 255

Lys Glu Thr Val Phe Thr Lys Ser Pro Tyr Gln Glu Phe Ser Asp His
 260 265 270

Leu Val Lys Thr His Thr Arg Val Ser Val Gln Arg Thr Gln Ala Pro
 275 280 285

Ala Val Ala Thr Thr
 290

<210> 10
 <211> 153
 <212> PRT
 <213> Cervus elaphus

<400> 10

Lys Ala Lys Lys Glu Ala Pro Ala Pro Pro Lys Ala Glu Ala Lys Ala
 1 5 10 15

Lys Ala Leu Lys Ala Lys Lys Ala Val Leu Lys Gly Val His Ser His
 20 25 30

Lys Lys Lys Lys Ile Arg Thr Ser Pro Thr Phe Arg Arg Pro Lys Thr
 35 40 45

Leu Arg Leu Arg Arg Gln Pro Lys Tyr Pro Arg Lys Ser Ala Pro Arg
 50 55 60

Arg Asn Lys Leu Asp His Tyr Ala Ile Ile Lys Phe Pro Leu Thr Thr
 65 70 75 80

Glu Ser Ala Met Lys Lys Ile Glu Asp Asn Asn Thr Leu Val Phe Ile
 85 90 95

Val Asp Val Lys Ala Asn Lys His Gln Ile Lys Gln Ala Val Lys Lys
 100 105 110

Leu Tyr Asp Ile Asp Val Ala Lys Val Asn Thr Leu Ile Arg Pro Asp
 115 120 125

Gly Glu Lys Lys Ala Tyr Val Arg Leu Ala Pro Asp Tyr Asp Ala Leu
 130 135 140

Asp Val Ala Asn Lys Ile Gly Ile Ile
 145 150

<210> 11
 <211> 156
 <212> PRT
 <213> Homo sapiens

<400> 11

Met Ala Pro Lys Ala Lys Lys Glu Ala Pro Ala Pro Pro Lys Ala Glu
 1 5 10 15

Ala Lys Ala Lys Ala Leu Lys Ala Lys Lys Ala Val Leu Lys Gly Val
 20 25 30

His Ser His Lys Lys Lys Lys Ile Arg Thr Ser Pro Thr Phe Arg Arg
 35 40 45

Pro Lys Thr Leu Arg Leu Arg Arg Gln Pro Lys Tyr Pro Arg Lys Ser
 50 55 60

Ala Pro Arg Arg Asn Lys Leu Asp His Tyr Ala Ile Ile Lys Phe Pro
 65 70 75 80

Leu Thr Thr Glu Ser Ala Met Lys Lys Ile Glu Asp Asn Asn Thr Leu
85 90 95

Val Phe Ile Val Asp Val Lys Ala Asn Lys His Gln Ile Lys Gln Ala
100 105 110

Val Lys Lys Leu Tyr Asp Ile Asp Val Ala Lys Val Asn Thr Leu Ile
115 120 125

Arg Pro Asp Gly Glu Lys Lys Ala Tyr Val Arg Leu Ala Pro Asp Tyr
130 135 140

Asp Ala Leu Asp Val Ala Asn Lys Ile Gly Ile Ile
145 150 155

<210> 12
<211> 156
<212> PRT
<213> Rattus rattus

<400> 12

Met Ala Pro Lys Ala Lys Lys Glu Ala Pro Ala Pro Pro Lys Ala Glu
1 5 10 15

Ala Lys Ala Lys Ala Leu Lys Ala Lys Lys Ala Val Leu Lys Gly Val
20 25 30

His Ser His Lys Lys Lys Lys Ile Arg Thr Ser Pro Thr Phe Arg Arg
35 40 45

Pro Lys Thr Leu Arg Leu Arg Arg Gln Pro Lys Tyr Pro Arg Lys Ser
50 55 60

Ala Pro Arg Arg Asn Lys Leu Asp His Tyr Ala Ile Ile Lys Phe Pro
65 70 75 80

Leu Thr Thr Glu Ser Ala Met Lys Lys Ile Glu Asp Asn Asn Thr Leu
85 90 95

Val Phe Ile Val Asp Val Lys Ala Asn Lys His Gln Ile Lys Gln Ala
100 105 110

Val Lys Lys Leu Tyr Asp Ile Asp Val Ala Lys Val Asn Thr Leu Ile
115 120 125

Arg Pro Asp Gly Glu Lys Lys Ala Tyr Val Arg Leu Ala Pro Asp Tyr
130 135 140

25/75

Asp Ala Leu Asp Val Ala Asn Lys Ile Gly Ile Ile
 145 150 155

<210> 13
 <211> 224
 <212> PRT
 <213> Cervus elaphus

<400> 13

Ala Ala Val Arg Leu Leu Ser Phe Ala Lys Ala Leu Gly Ala Pro Arg
 1 5 10 15

Pro Ser Gly Thr Arg Leu Ser Pro Ala Pro Pro Pro Arg Cys Pro Arg
 20 25 30

Gly Arg Ser Ala Pro Pro Arg Gly Arg Arg Arg Arg Ser Pro Arg Gly
 35 40 45

Asp Arg Arg Gly Cys Gln Gln Asn Arg Leu Leu Gln Lys Trp Lys Arg
 50 55 60

Ser Gln Lys Arg Arg Arg Glu Arg Ile Asn Leu Gln Thr Lys Lys Cys
 65 70 75 80

Lys Gln Lys Gly Lys Glu Glu Gln Arg Glu Asn Arg Arg Lys Trp Pro
 85 90 95

Thr Lys Arg Leu Lys Lys Thr Cys Leu Gln Lys Met Glu Arg Leu Lys
 100 105 110

Thr Arg Arg Ala Gln Pro Leu Met Lys Gln Lys Arg Lys Lys Pro Ser
 115 120 125

Leu Ile Asn Asn His Thr Leu Ser Pro Val Ser Gly Pro Cys Phe Pro
 130 135 140

Ser Cys Thr Ile Gln Arg Asn Ile Phe Ile Asn Tyr Phe Val Asn Ala
 145 150 155 160

Ser Phe Leu Val Ala Leu Glu Thr Phe Leu Lys Arg Arg Glu Ser His
 165 170 175

Leu Ile Pro Phe Phe Lys Cys Lys Cys Phe Phe Leu Arg Gly Glu Ile
 180 185 190

Ile Cys Trp Val Gly Tyr Phe Leu Val Gln Pro Glu Asn Ser Gly Ile
 195 200 205

Leu Asp Met Gly Gly Phe Asp Cys Leu Gly Cys Gln Leu Asn Ile Pro
 210 215 220

<210> 14
 <211> 168
 <212> PRT
 <213> Homo sapiens

<400> 14

Met Val Leu Phe Phe Arg Ile Asn Leu Gln Thr Lys Lys Cys Lys Gln
 1 5 10 15

Lys Gly Lys Gly Glu Gln Arg Glu Asn Arg Pro Lys Trp Leu Thr Lys
 20 25 30

Lys Leu Lys Lys Thr Tyr Leu Arg Lys Thr Gly Lys Arg Arg Leu Arg
 35 40 45

Arg Val Gln Pro Leu Met Lys Gln Glu Arg Lys Lys Pro Ser Leu Ile
 50 55 60

Asn Asn His Ile Pro Cys Leu Ile Ser Gly Pro Cys Leu Pro Ser Cys
 65 70 75 80

Thr Ile Gln Arg Asn Ile Phe Ile Asn Tyr Phe Val Asn Ala Ser Phe
 85 90 95

Leu Val Ala Leu Glu Thr Phe Leu Arg Arg Arg Glu Ser His Leu Ile
 100 105 110

Pro Phe Phe Lys Cys Lys Cys Phe Phe Leu Arg Gly Glu Ile Ile Cys
 115 120 125

Trp Leu Phe Ile Phe Trp Tyr Asn Gln Lys Ile Val Trp Asp Ile Glu
 130 135 140

Leu Trp Glu Ala Leu Thr Val Ser Gly Val Ser Leu Thr Phe His Arg
 145 150 155 160

Trp Gly Val Ser Phe Tyr Ile Leu
 165

<210> 15
 <211> 215
 <212> PRT
 <213> Cervus elaphus

<400> 15

Ser Glu Gln Leu Val Arg His Phe Leu Ile Glu Thr Gly Pro Lys Gly
1 5 10 15

Val Lys Ile Lys Gly Cys Pro Ser Glu Pro Tyr Phe Gly Ser Leu Ser
20 25 30

Ala Leu Val Ser Gln His Ser Ile Ser Pro Leu Ser Leu Pro Cys Cys
35 40 45

Leu Arg Ile Pro Ser Lys Asp Pro Leu Glu Glu Val Pro Glu Ala Pro
50 55 60

Val Pro Ser Asn Met Ser Thr Ala Ala Asp Leu Leu Arg Gln Gly Ala
65 70 75 80

Ala Cys Ser Val Leu Tyr Leu Thr Ser Val Glu Thr Glu Ser Leu Thr
85 90 95

Gly Pro Gln Ala Val Ala Arg Ala Ser Ser Ala Ala Leu Ser Cys Ser
100 105 110

Pro Arg Pro Thr Pro Ala Val Val His Phe Lys Val Ser Ala Gln Gly
115 120 125

Ile Thr Leu Thr Asp Asn Gln Arg Lys Leu Phe Phe Arg Arg His Tyr
130 135 140

Pro Val Asn Ser Ile Thr Phe Ser Ser Thr Asp Pro Gln Asp Arg Arg
145 150 155 160

Trp Thr Asn Ser Asp Gly Thr Thr Ser Lys Ile Phe Gly Phe Val Ala
165 170 175

Lys Lys Pro Gly Ser Pro Trp Glu Asn Val Cys His Leu Phe Ala Glu
180 185 190

Leu Asp Pro Asp Gln Pro Ala Gly Ala Ile Val Thr Phe Ile Thr Lys
195 200 205

Val Leu Leu Gly Gln Arg Lys
210 215

<210> 16
<211> 1285
<212> PRT
<213> Homo sapiens

28/75

<400> 16

Met Glu Arg Arg Trp Asp Leu Asp Leu Thr Tyr Val Thr Glu Arg Ile
 1 5 10 15

Leu Ala Ala Ala Phe Pro Ala Arg Pro Asp Glu Gln Arg His Arg Gly
 20 25 30

His Leu Arg Glu Leu Ala His Val Leu Gln Ser Lys His Arg Asp Lys
 35 40 45

Tyr Leu Leu Phe Asn Leu Ser Glu Lys Arg His Asp Leu Thr Arg Leu
 50 55 60

Asn Pro Lys Val Gln Asp Phe Gly Trp Pro Glu Leu His Ala Pro Pro
 65 70 75 80

Leu Asp Lys Leu Cys Ser Ile Cys Lys Ala Met Glu Thr Trp Leu Ser
 85 90 95

Ala Asp Pro Gln His Val Val Val Leu Tyr Cys Lys Gly Asn Lys Gly
 100 105 110

Lys Leu Gly Val Ile Val Ser Ala Tyr Met His Tyr Ser Lys Ile Ser
 115 120 125

Ala Gly Ala Asp Gln Ala Leu Ala Thr Leu Thr Met Arg Lys Phe Cys
 130 135 140

Glu Asp Lys Val Ala Thr Glu Leu Gln Pro Ser Gln Arg Arg Tyr Ile
 145 150 155 160

Ser Tyr Phe Ser Gly Leu Leu Ser Gly Ser Ile Arg Met Asn Ser Ser
 165 170 175

Pro Leu Phe Leu His Tyr Val Leu Ile Pro Met Leu Pro Ala Phe Glu
 180 185 190

Pro Gly Thr Gly Phe Gln Pro Phe Leu Lys Ile Tyr Gln Ser Met Gln
 195 200 205

Leu Val Tyr Thr Ser Gly Val Tyr His Ile Ala Gly Pro Gly Pro Gln
 210 215 220

Gln Leu Cys Ile Ser Leu Glu Pro Ala Leu Leu Leu Lys Gly Asp Val
 225 230 235 240

29/75

Met Val Thr Cys Tyr His Lys Gly Gly Arg Gly Thr Asp Arg Thr Leu
245 250 255

Val Phe Arg Val Gln Phe His Thr Cys Thr Ile His Gly Pro Gln Leu
260 265 270

Thr Phe Pro Lys Asp Gln Leu Asp Glu Ala Trp Thr Asp Glu Arg Phe
275 280 285

Pro Phe Gln Ala Ser Val Glu Phe Val Phe Ser Ser Ser Pro Glu Lys
290 295 300

Ile Lys Gly Ser Thr Pro Arg Asn Asp Pro Ser Val Ser Val Asp Tyr
305 310 315 320

Asn Thr Thr Glu Pro Ala Val Arg Trp Asp Ser Tyr Glu Asn Phe Asn
325 330 335

Gln His His Glu Asp Ser Val Asp Gly Ser Leu Thr His Thr Arg Gly
340 345 350

Pro Leu Asp Gly Ser Pro Tyr Ala Gln Val Gln Arg Pro Pro Arg Gln
355 360 365

Thr Pro Pro Ala Pro Ser Pro Glu Pro Pro Pro Pro Pro Met Leu Ser
370 375 380

Val Ser Ser Asp Ser Gly His Ser Ser Thr Leu Thr Thr Glu Pro Ala
385 390 395 400

Ala Glu Ser Pro Gly Arg Pro Pro Pro Thr Ala Ala Glu Arg Gln Glu
405 410 415

Leu Asp Arg Leu Leu Gly Gly Cys Gly Val Ala Ser Gly Gly Arg Gly
420 425 430

Ala Gly Arg Glu Thr Ala Ile Leu Asp Asp Glu Glu Gln Pro Thr Val
435 440 445

Gly Gly Gly Pro His Leu Gly Val Tyr Pro Gly His Arg Pro Gly Leu
450 455 460

Ser Arg His Cys Ser Cys Arg Gln Gly Tyr Arg Glu Pro Cys Gly Val
465 470 475 480

Pro Asn Gly Gly Tyr Tyr Arg Pro Glu Gly Thr Leu Glu Arg Arg Arg
 485 490 495

Leu Ala Tyr Gly Gly Tyr Glu Gly Ser Pro Gln Gly Tyr Ala Glu Ala
 500 505 510

Ser Met Glu Lys Arg Arg Leu Cys Arg Ser Leu Ser Glu Gly Leu Tyr
 515 520 525

Pro Tyr Pro Pro Glu Met Gly Lys Pro Ala Thr Gly Asp Phe Gly Tyr
 530 535 540

Arg Ala Pro Gly Tyr Arg Glu Val Val Ile Leu Glu Asp Pro Gly Leu
 545 550 555 560

Pro Ala Leu Tyr Pro Cys Pro Ala Cys Glu Glu Lys Leu Ala Leu Pro
 565 570 575

Thr Ala Ala Leu Tyr Gly Leu Arg Leu Glu Arg Glu Ala Gly Glu Gly
 580 585 590

Trp Ala Ser Glu Ala Gly Lys Pro Leu Leu His Pro Val Arg Pro Gly
 595 600 605

His Pro Leu Pro Leu Leu Leu Pro Ala Cys Gly His His His Ala Pro
 610 615 620

Met Pro Asp Tyr Ser Cys Leu Lys Pro Pro Lys Ala Gly Glu Glu Gly
 625 630 635 640

His Glu Gly Cys Ser Tyr Thr Met Cys Pro Glu Gly Arg Tyr Gly His
 645 650 655

Pro Gly Tyr Pro Ala Leu Val Thr Tyr Ser Tyr Gly Gly Ala Val Pro
 660 665 670

Ser Tyr Cys Pro Ala Tyr Gly Arg Val Pro His Ser Cys Gly Ser Pro
 675 680 685

Gly Glu Gly Arg Gly Tyr Pro Ser Pro Gly Ala His Ser Pro Arg Ala
 690 695 700

Gly Ser Ile Ser Pro Gly Ser Pro Pro Tyr Pro Gln Ser Arg Lys Leu
 705 710 715 720

Ser Tyr Glu Ile Pro Thr Glu Glu Gly Gly Asp Arg Tyr Pro Leu Pro
725 730 735

Gly His Leu Ala Ser Ala Gly Pro Leu Ala Ser Ala Glu Ser Leu Glu
740 745 750

Pro Val Ser Trp Arg Glu Gly Pro Ser Gly His Ser Thr Leu Pro Arg-
755 760 765

Ser Pro Arg Asp Ala Pro Cys Ser Ala Ser Ser Glu Leu Ser Gly Pro
770 775 780

Ser Thr Pro Leu His Thr Ser Ser Pro Val Gln Gly Lys Glu Ser Thr
785 790 795 800

Arg Arg Gln Asp Thr Arg Ser Pro Thr Ser Ala Pro Thr Gln Arg Leu
805 810 815

Ser Pro Gly Glu Ala Leu Pro Pro Val Ser Gln Ala Gly Thr Gly Lys
820 825 830

Ala Pro Glu Leu Pro Ser Gly Ser Gly Pro Glu Pro Leu Ala Pro Ser
835 840 845

Pro Val Ser Pro Thr Phe Pro Pro Ser Ser Pro Ser Asp Trp Pro Gln
850 855 860

Glu Arg Ser Pro Gly Gly His Ser Asp Gly Ala Ser Pro Arg Ser Pro
865 870 875 880

Val Pro Thr Thr Leu Pro Gly Leu Arg His Ala Pro Trp Gln Gly Pro
885 890 895

Arg Gly Pro Pro Asp Ser Pro Asp Gly Ser Pro Leu Thr Pro Val Pro
900 905 910

Ser Gln Met Pro Trp Leu Val Ala Ser Pro Glu Pro Pro Gln Ser Ser
915 920 925

Pro Thr Pro Ala Phe Pro Leu Ala Ala Ser Tyr Asp Thr Asn Gly Leu
930 935 940

Ser Gln Pro Pro Leu Pro Glu Lys Arg His Leu Pro Gly Pro Gly Gln
945 950 955 960

Gln Pro Gly Pro Trp Gly Pro Glu Gln Ala Ser Ser Pro Ala Arg Gly
 965 970 975

Ile Ser His His Val Thr Phe Ala Pro Leu Leu Ser Asp Asn Val Pro
 980 985 990

Gln Thr Pro Glu Pro Pro Thr Gln Glu Ser Gln Ser Asn Val Lys Phe
 995 1000 1005

Val Gln Asp Thr Ser Lys Phe Trp Tyr Lys Pro His Leu Ser Arg
 1010 1015 1020

Asp Gln Ala Ile Ala Leu Leu Lys Asp Lys Asp Pro Gly Ala Phe
 1025 1030 1035

Leu Ile Arg Asp Ser His Ser Phe Gln Gly Ala Tyr Gly Leu Ala
 1040 1045 1050

Leu Lys Val Ala Thr Pro Pro Pro Ser Ala Gln Pro Trp Lys Gly
 1055 1060 1065

Asp Pro Val Glu Gln Leu Val Arg His Phe Leu Ile Glu Thr Gly
 1070 1075 1080

Pro Lys Gly Val Lys Ile Lys Gly Cys Pro Ser Glu Pro Tyr Phe
 1085 1090 1095

Gly Ser Leu Ser Ala Leu Val Ser Gln His Ser Ile Ser Pro Ile
 1100 1105 1110

Ser Leu Pro Cys Cys Leu Arg Ile Pro Ser Lys Asp Pro Leu Glu
 1115 1120 1125

Glu Thr Pro Glu Ala Pro Val Pro Thr Asn Met Ser Thr Ala Ala
 1130 1135 1140

Asp Leu Leu Arg Gln Gly Ala Ala Cys Ser Val Leu Tyr Leu Thr
 1145 1150 1155

Ser Val Glu Thr Glu Ser Leu Thr Gly Pro Gln Ala Val Ala Arg
 1160 1165 1170

Ala Ser Ser Ala Ala Leu Ser Cys Ser Pro Arg Pro Thr Pro Ala
 1175 1180 1185

Val Val His Phe Lys Val Ser Ala Gln Gly Ile Thr Leu Thr Asp
1190 1195 1200

Asn Gln Arg Lys Leu Phe Phe Arg Arg His Tyr Pro Val Asn Ser
1205 1210 1215

Ile Thr Phe Ser Ser Thr Asp Pro Gln Asp Arg Arg Trp Thr Asn
1220 1225 1230

Pro Asp Gly Thr Thr Ser Lys Ile Phe Gly Phe Val Ala Lys Lys
1235 1240 1245

Pro Gly Ser Pro Trp Glu Asn Val Cys His Leu Phe Ala Glu Leu
1250 1255 1260

Asp Pro Asp Gln Pro Ala Gly Ala Ile Val Thr Phe Ile Thr Lys
1265 1270 1275

Val Leu Leu Gly Gln Arg Lys
1280 1285

<210> 17
<211> 303
<212> PRT
<213> Homo sapiens

<400> 17

Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
1 5 10 15

Ala Ala Pro Gln Gln Glu Ala Leu Pro Asp Glu Thr Glu Val Val Glu
20 25 30

Glu Thr Val Ala Glu Val Thr Glu Val Ser Val Gly Ala Asn Pro Val
35 40 45

Gln Val Glu Val Gly Glu Phe Asp Asp Gly Ala Glu Glu Thr Glu Glu
50 55 60

Glu Val Val Ala Glu Asn Pro Cys Gln Asn His His Cys Lys His Gly
65 70 75 80

Lys Val Cys Glu Leu Asp Glu Asn Asn Thr Pro Met Cys Val Cys Gln
85 90 95

Asp Pro Thr Ser Cys Pro Ala Pro Ile Gly Glu Phe Glu Lys Val Cys
100 105 110

Ser Asn Asp Asn Lys Thr Phe Asp Ser Ser Cys His Phe Phe Ala Thr
 115 120 125

Lys Cys Thr Leu Glu Gly Thr Lys Lys Gly His Lys Leu His Leu Asp
 130 135 140

Tyr Ile Gly Pro Cys Lys Tyr Ile Pro Pro Cys Leu Asp Ser Glu Leu
 145 150 155 160

Thr Glu Phe Pro Leu Arg Met Arg Asp Trp Leu Lys Asn Val Leu Val
 165 170 175

Thr Leu Tyr Glu Arg Asp Glu Asp Asn Asn Leu Leu Thr Glu Lys Gln
 180 185 190

Lys Leu Arg Val Lys Lys Ile His Glu Asn Glu Lys Arg Leu Glu Ala
 195 200 205

Gly Asp His Pro Val Glu Leu Leu Ala Arg Asp Phe Glu Lys Asn Tyr
 210 215 220

Asn Met Tyr Ile Phe Pro Val His Trp Gln Phe Gly Gln Leu Asp Gln
 225 230 235 240

His Pro Ile Asp Gly Tyr Leu Ser His Thr Glu Leu Ala Pro Leu Arg
 245 250 255

Ala Pro Leu Ile Pro Met Glu His Cys Thr Thr Arg Phe Phe Glu Thr
 260 265 270

Cys Asp Leu Asp Asn Asp Lys Tyr Ile Ala Leu Asp Glu Trp Ala Gly
 275 280 285

Cys Phe Gly Ile Lys Gln Lys Asp Ile Asp Lys Asp Leu Val Ile
 290 295 300

<210> 18

<211> 136

<212> PRT

<213> Cervus elaphus

<400> 18

Arg Arg Arg Ser Arg Met Glu Ile Pro Val Pro Val Gln Pro Ser Trp
 1 5 10 15

Leu Arg Arg Ala Ser Ala Pro Leu Pro Gly Leu Ser Ala Pro Gly Arg
 20 25 30

Leu Phe Asp Gln Arg Phe Gly Glu Gly Leu Leu Glu Ala Glu Leu Ala
 35 40 45

Ala Leu Cys Pro Ala Ala Leu Ala Pro Tyr Tyr Leu Arg Ala Pro Ser
 50 55 60

Val Ala Leu Pro Thr Ala Gln Val Ser Thr Asp Pro Gly His Phe Ser
 65 70 75 80

Val Leu Leu Asp Val Lys His Phe Ser Pro Glu Glu Ile Ala Val Lys
 85 90 95

Val Val Gly Asp His Val Glu Val His Ala Arg His Glu Glu Arg Pro
 100 105 110

Asp Glu His Gly Tyr Ile Ala Arg Glu Phe Thr Arg Leu Pro Leu Ala
 115 120 125

Ala Gly Val Asp Pro Ala Ala Val
 130 135

<210> 19
 <211> 160
 <212> PRT
 <213> Homo sapiens

<400> 19

Met Glu Ile Pro Val Pro Val Gln Pro Ser Trp Leu Arg Arg Ala Ser
 1 5 10 15

Ala Pro Leu Pro Gly Leu Ser Ala Pro Gly Arg Leu Phe Asp Gln Arg
 20 25 30

Phe Gly Glu Gly Leu Leu Glu Ala Glu Leu Ala Ala Leu Cys Pro Thr
 35 40 45

Thr Leu Ala Pro Tyr Tyr Leu Arg Ala Pro Ser Val Ala Leu Pro Val
 50 55 60

Ala Gln Val Pro Thr Asp Pro Gly His Phe Ser Val Leu Leu Asp Val
 65 70 75 80

Lys His Phe Ser Pro Glu Glu Ile Ala Val Lys Val Val Gly Glu His
 85 90 95

Val Glu Val His Ala Arg His Glu Glu Arg Pro Asp Glu His Gly Phe
 100 105 110

Val Ala Arg Glu Phe His Arg Arg Tyr Arg Leu Pro Pro Gly Val Asp
 115 120 125

Pro Ala Ala Val Thr Ser Ala Leu Ser Pro Glu Gly Val Leu Ser Ile
 130 135 140

Gln Ala Ala Pro Ala Ser Ala Gln Ala Pro Pro Pro Ala Ala Ala Lys
 145 150 155 160

<210> 20
 <211> 162
 <212> PRT
 <213> Rattus norvegicus

<400> 20

Met Glu Ile Arg Val Pro Val Gln Pro Ser Trp Leu Arg Arg Ala Ser
 1 5 10 15

Ala Pro Leu Pro Gly Phe Ser Thr Pro Gly Arg Leu Phe Asp Gln Arg
 20 25 30

Phe Gly Glu Gly Leu Leu Glu Ala Glu Leu Ala Ser Leu Cys Pro Ala
 35 40 45

Ala Ile Ala Pro Tyr Tyr Leu Arg Ala Pro Ser Val Ala Leu Pro Thr
 50 55 60

Ala Gln Val Pro Thr Asp Pro Gly Tyr Phe Ser Val Leu Leu Asp Val
 65 70 75 80

Lys His Phe Ser Pro Glu Glu Ile Ser Val Lys Val Val Gly Asp His
 85 90 95

Val Glu Val His Ala Arg His Glu Glu Arg Pro Asp Glu His Gly Phe
 100 105 110

Ile Ala Arg Glu Phe His Arg Arg Tyr Arg Leu Pro Pro Gly Val Asp
 115 120 125

Pro Ala Ala Val Thr Ser Ala Leu Ser Pro Glu Gly Val Leu Ser Ile
 130 135 140

Gln Ala Thr Pro Ala Ser Ala Gln Ala Ser Leu Pro Ser Pro Pro Ala
 145 150 155 160

Ala Lys

<210> 21
 <211> 363
 <212> PRT
 <213> Cervus elaphus

<400> 21

Gly Asp Arg Gly Gln Lys Gly His Arg Gly Phe Thr Gly Leu Gln Gly
 1 5 10 15

Leu Pro Gly Pro Pro Gly Pro Asn Gly Glu Gln Gly Ser Ala Gly Ile
 20 25 30

Pro Gly Pro Phe Gly Pro Arg Gly Pro Pro Gly Pro Val Gly Pro Ser
 35 40 45

Gly Lys Glu Gly Ser Pro Gly Pro Leu Gly Pro Ile Gly Pro Pro Gly
 50 55 60

Val Arg Gly Ser Val Gly Glu Ala Gly Pro Glu Gly Pro Pro Gly Glu
 65 70 75 80

Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly His Leu Thr Ala Ala
 85 90 95

Leu Gly Asp Ile Met Gly His Tyr Asp Glu Ser Met Pro Asp Pro Leu
 100 105 110

Pro Glu Phe Thr Glu Asp Gln Ala Ala Pro Asp Asp Lys Asn Lys Thr
 115 120 125

Asp Pro Gly Val His Ala Thr Leu Lys Ser Leu Ser Ser Gln Ile Glu
 130 135 140

Thr Met Arg Ser Pro Asp Gly Ser Arg Lys His Pro Ala Arg Thr Cys
 145 150 155 160

Asp Asp Leu Lys Leu Cys His Ser Ala Lys Gln Ser Gly Glu Tyr Trp
 165 170 175

Ile Asp Pro Asn Gln Gly Ser Ala Glu Asp Ala Ile Lys Val Tyr Cys
 180 185 190

Asn Met Glu Thr Gly Glu Thr Cys Ile Ser Ala Asn Pro Ser Ser Val
 195 200 205

Pro Arg Lys Thr Trp Trp Ala Ser Lys Ser Pro Asp Asn Lys Pro Val
 210 215 220

Trp Tyr Gly Leu Asp Met Asn Arg Gly Ser Gln Phe Val Tyr Gly Asp
225 230 235 240

His Gln Ser Pro Asn Ala Ala Ile Thr Gln Met Thr Phe Leu Arg Leu
245 250 255

Leu Ser Lys Glu Ala Ser Gln Asn Ile Thr Tyr Ile Cys Lys Asn Ser
260 265 270

Val Gly Tyr Met Asp Asp Gln Thr Lys Asn Leu Lys Lys Ala Val Val
275 280 285

Leu Lys Gly Ser Asn Asp Leu Glu Ile Lys Ala Glu Gly Asn Val Arg
290 295 300

Phe Arg Tyr Ile Val Leu His Asp Ser Cys Ser Lys Arg Asn Gly Asn
305 310 315 320

Val Gly Lys Thr Ile Phe Glu Tyr Arg Thr Gln Asn Val Ala Arg Leu
325 330 335

Pro Ile Ile Asp Leu Ala Pro Val Asp Val Gly Ser Thr Asp Gln Glu
340 345 350

Phe Gly Ile Glu Ile Gly Pro Val Cys Phe Val
355 360

<210> 22
<211> 1496
<212> PRT
<213> Homo sapiens

<400> 22

Met Met Ala Asn Trp Ala Glu Ala Arg Pro Leu Leu Ile Leu Ile Val
1 5 10 15

Leu Leu Gly Gln Phe Val Ser Ile Lys Ala Gln Glu Glu Asp Glu Asp
20 25 30

Glu Gly Tyr Gly Glu Glu Ile Ala Cys Thr Gln Asn Gly Gln Met Tyr
35 40 45

Leu Asn Arg Asp Ile Trp Lys Pro Ala Pro Cys Gln Ile Cys Val Cys
50 55 60

Asp Asn Gly Ala Ile Leu Cys Asp Lys Ile Glu Cys Gln Asp Val Leu
65 70 75 80

Ser Gln Thr Pro Gly Gly Gly Asn Thr Asn Phe Gly Arg Gly Arg Lys
100 105 110

Gly Gln Lys Gly Glu Pro Gly Leu Val Pro Val Val Thr Gly Ile Arg _
115 120 125

Gly Arg Pro Gly Pro Ala Gly Pro Pro Gly Ser Gln Gly Pro Arg Gly
130 135 140

Glu Arg Gly Pro Lys Gly Arg Pro Gly Pro Arg Gly Pro Gln Gly Ile
145 150 155 160

Asp Gly Glu Pro Gly Val Pro Gly Gln Pro Gly Ala Pro Gly Pro Pro
165 170 175

Gly His Pro Ser His Pro Gly Pro Asp Gly Leu Ser Arg Pro Phe Ser
180 185 190

Ala Gln Met Ala Gly Leu Asp Glu Lys Ser Gly Leu Gly Ser Gln Val
195 200 205

Gly Leu Met Pro Gly Ser Val Gly Pro Val Gly Pro Arg Gly Pro Gln
210 215 220

Gly Leu Gln Gly Gln Gln Gly Gly Ala Gly Pro Thr Gly Pro Pro Gly
225 230 235 240

Glu Pro Gly Asp Pro Gly Pro Met Gly Pro Ile Gly Ser Arg Gly Pro
245 250 255

Glu Gly Pro Pro Gly Lys Pro Gly Glu Asp Gly Glu Pro Gly Arg Asn
260 265 270

Gly Asn Pro Gly Glu Val Gly Phe Ala Gly Ser Pro Gly Ala Arg Gly
275 280 285

Phe Pro Gly Ala Pro Gly Leu Pro Gly Leu Lys Gly His Arg Gly His
290 295 300

Lys Gly Leu Glu Gly Pro Lys Gly Glu Val Gly Ala Pro Gly Ser Lys
305 310 315 320

Gly Glu Ala Gly Pro Thr Gly Pro Met Gly Ala Met Gly Pro Leu Gly
325 330 335

Pro Arg Gly Met Pro Gly Glu Arg Gly Arg Leu Gly Pro Gln Gly Ala
340 345 350

Pro Gly Gln Arg Gly Ala His Gly Met Pro Gly Lys Pro Gly Pro Met
355 360 365

Gly Pro Leu Gly Ile Pro Gly Ser Ser Gly Phe Pro Gly Asn Pro Gly
370 375 380

Met Lys Gly Glu Ala Gly Pro Thr Gly Ala Arg Gly Pro Glu Gly Pro
385 390 395 400

Gln Gly Gln Arg Gly Glu Thr Gly Pro Pro Gly Pro Val Gly Ser Pro
405 410 415

Gly Leu Pro Gly Ala Ile Gly Thr Asp Gly Thr Pro Gly Pro Lys Gly
420 425 430

Pro Thr Gly Ser Pro Gly Thr Ser Gly Pro Pro Gly Ser Ala Gly Pro
435 440 445

Pro Gly Ser Pro Gly Pro Gln Gly Ser Thr Gly Pro Gln Gly Asn Ser
450 455 460

Gly Leu Pro Gly Asp Pro Gly Phe Lys Gly Glu Ala Gly Pro Lys Gly
465 470 475 480

Glu Pro Gly Pro His Gly Ile Gln Gly Pro Ile Gly Pro Pro Gly Glu
485 490 495

Glu Gly Lys Arg Gly Pro Arg Gly Asp Pro Gly Thr Leu Gly Pro Pro
500 505 510

Gly Pro Val Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe Pro Gly
515 520 525

Ser Asp Gly Leu Pro Gly Pro Lys Gly Ala Gln Gly Glu Arg Gly Pro
530 535 540

Val Gly Ser Ser Gly Pro Lys Gly Ser Gln Gly Asp Pro Gly Arg Pro
545 550 555 560

Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Asn Pro Gly
 565 570 575

Val Gln Gly Pro Glu Gly Lys Leu Gly Pro Leu Gly Ala Pro Gly Glu
 580 585 590

Asp Gly Arg Pro Gly Pro Pro Gly Ser Ile Gly Ile Lys Gly Gln Pro
 595 600 605

Gly Thr Met Gly Leu Pro Gly Pro Lys Gly Ser Asn Gly Asp Pro Gly
 610 615 620

Lys Pro Gly Glu Ala Gly Asn Pro Gly Val Pro Gly Gln Arg Gly Ala
 625 630 635 640

Pro Gly Lys Asp Gly Lys Val Gly Pro Tyr Gly Pro Pro Gly Pro Pro
 645 650 655

Gly Leu Arg Gly Glu Arg Gly Glu Gln Gly Pro Pro Gly Pro Thr Gly
 660 665 670

Phe Gln Gly His Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly Gly Lys
 675 680 685

Pro Gly Asp Gln Gly Val Pro Gly Gly Pro Gly Ala Val Gly Pro Leu
 690 695 700

Gly Pro Arg Gly Glu Arg Gly Asn Pro Gly Glu Arg Gly Glu Pro Gly
 705 710 715 720

Ile Thr Gly Leu Pro Gly Glu Lys Gly Met Ala Gly Gly His Gly Pro
 725 730 735

Asp Gly Pro Lys Gly Ser Pro Gly Pro Ser Gly Thr Pro Gly Asp Thr
 740 745 750

Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ile Ala Gly
 755 760 765

Thr Pro Gly Pro Lys Gly Asp Arg Gly Gly Ile Gly Glu Lys Gly Ala
 770 775 780

Glu Gly Thr Ala Gly Asn Asp Gly Ala Gly Gly Leu Pro Gly Pro Leu
 785 790 795 800

Gly Pro Pro Gly Pro Ala Gly Leu Leu Gly Glu Lys Gly Glu Pro Gly
805 810 815

Pro Arg Gly Leu Val Gly Pro Pro Gly Ser Arg Gly Asn Pro Gly Ser
820 825 830

Arg Gly Glu Asn Gly Pro Thr Gly Ala Val Gly Phe Ala Gly Pro Gln
835 840 845

Gly Ser Asp Gly Gln Pro Gly Val Lys Gly Glu Pro Gly Glu Pro Gly
850 855 860

Gln Lys Gly Asp Ala Gly Ser Pro Gly Pro Gln Gly Leu Ala Gly Ser
865 870 875 880

Pro Gly Pro His Gly Pro Asn Gly Val Pro Gly Leu Lys Gly Gly Arg
885 890 895

Gly Thr Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ser Ala Gly
900 905 910

Arg Val Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro Ala Gly Pro
915 920 925

Leu Gly Glu Pro Gly Lys Glu Gly Pro Pro Gly Pro Arg Gly Asp Pro
930 935 940

Gly Ser His Gly Arg Val Gly Val Arg Gly Pro Ala Gly Pro Pro Gly
945 950 955 960

Gly Pro Gly Asp Lys Gly Asp Pro Gly Glu Asp Gly Gln Pro Gly Pro
965 970 975

Asp Gly Pro Pro Gly Pro Ala Gly Thr Thr Gly Gln Arg Gly Ile Val
980 985 990

Gly Met Pro Gly Gln Arg Gly Glu Arg Gly Met Pro Gly Leu Pro Gly
995 1000 1005

Pro Ala Gly Thr Pro Gly Lys Val Gly Pro Thr Gly Ala Thr Gly
1010 1015 1020

Asp Lys Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Ser Asn Gly
1025 1030 1035

Pro Val Gly Glu Pro Gly Pro Glu Gly Pro Ala Gly Asn Asp Gly
 1040 1045 1050

Thr Pro Gly Arg Asp Gly Ala Val Gly Glu Arg Gly Asp Arg Gly
 1055 1060 1065

Asp Pro Gly Pro Ala Gly Leu Pro Gly Ser Gln Gly Ala Pro Gly
 1070 1075 1080

Thr Pro Gly Pro Val Gly Ala Pro Gly Asp Ala Gly Gln Arg Gly
 1085 1090 1095

Asp Pro Gly Ser Arg Gly Pro Ile Gly His Leu Gly Arg Ala Gly
 1100 1105 1110

Lys Arg Gly Leu Pro Gly Pro Gln Gly Pro Arg Gly Asp Lys Gly
 1115 1120 1125

Asp His Gly Asp Arg Gly Asp Arg Gly Gln Lys Gly His Arg Gly
 1130 1135 1140

Phe Thr Gly Leu Gln Gly Leu Pro Gly Pro Pro Gly Pro Asn Gly
 1145 1150 1155

Glu Gln Gly Ser Ala Gly Ile Pro Gly Pro Phe Gly Pro Arg Gly
 1160 1165 1170

Pro Pro Gly Pro Val Gly Pro Ser Gly Lys Glu Gly Asn Pro Gly
 1175 1180 1185

Pro Leu Gly Pro Leu Gly Pro Pro Gly Val Arg Gly Ser Val Gly
 1190 1195 1200

Glu Ala Gly Pro Glu Gly Pro Pro Gly Glu Pro Gly Pro Pro Gly
 1205 1210 1215

Pro Pro Gly Pro Pro Gly His Leu Thr Ala Ala Leu Gly Asp Ile
 1220 1225 1230

Met Gly His Tyr Asp Glu Ser Met Pro Asp Pro Leu Pro Glu Phe
 1235 1240 1245

Thr Glu Asp Gln Ala Ala Pro Asp Asp Lys Asn Lys Thr Asp Pro
 1250 1255 1260

Gly Val His Ala Thr Leu Lys Ser Leu Ser Ser Gln Ile Glu Thr
 1265 1270 1275
 Met Arg Ser Pro Asp Gly Ser Lys Lys His Pro Ala Arg Thr Cys
 1280 1285 1290
 Asp Asp Leu Lys Leu Cys His Ser Ala Lys Gln Ser Gly Glu Tyr
 1295 1300 1305
 Trp Ile Asp Pro Asn Gln Gly Ser Val Glu Asp Ala Ile Lys Val
 1310 1315 1320
 Tyr Cys Asn Met Glu Thr Gly Glu Thr Cys Ile Ser Ala Asn Pro
 1325 1330 1335
 Ser Ser Val Pro Arg Lys Thr Trp Trp Ala Ser Lys Ser Pro Asp
 1340 1345 1350
 Asn Lys Pro Val Trp Tyr Gly Leu Asp Met Asn Arg Gly Ser Gln
 1355 1360 1365
 Phe Ala Tyr Gly Asp His Gln Ser Pro Asn Thr Ala Ile Thr Gln
 1370 1375 1380
 Met Thr Phe Leu Arg Leu Leu Ser Lys Glu Ala Ser Gln Asn Ile
 1385 1390 1395
 Thr Tyr Ile Cys Lys Asn Ser Val Gly Tyr Met Asp Asp Gln Ala
 1400 1405 1410
 Lys Asn Leu Lys Lys Ala Val Val Leu Lys Gly Ala Asn Asp Leu
 1415 1420 1425
 Asp Ile Lys Ala Glu Gly Asn Ile Arg Phe Arg Tyr Ile Val Leu
 1430 1435 1440
 Gln Asp Thr Cys Ser Lys Arg Asn Gly Asn Val Gly Lys Thr Val
 1445 1450 1455
 Phe Glu Tyr Arg Thr Gln Asn Val Ala Arg Leu Pro Ile Ile Asp
 1460 1465 1470
 Leu Ala Pro Val Asp Val Gly Gly Thr Asp Gln Glu Phe Gly Val
 1475 1480 1485

Glu Ile Gly Pro Val Cys Phe Val
1490 1495

<210> 23
<211> 1497
<212> PRT
<213> Mus musculus

<400> 23

Met Met Ala Asn Trp Val Gly Ala Arg Pro Leu Leu Ile Leu Ser Val
1 5 10 15

Leu Leu Gly Tyr Cys Val Ser Ile Lys Ala Gln Glu Gln Glu Asn Asp
20 25 30

Glu Tyr Asp Glu Glu Ile Ala Cys Thr Gln His Gly Gln Met Tyr Leu
35 40 45

Asn Arg Asp Ile Trp Lys Pro Ser Pro Cys Gln Ile Cys Val Cys Asp
50 55 60

Asn Gly Ala Ile Leu Cys Asp Lys Ile Glu Cys Pro Glu Val Leu Asn
65 70 75 80

Cys Ala Asn Pro Ile Thr Pro Pro Gly Glu Cys Cys Pro Val Cys Pro
85 90 95

Gln Thr Gly Gly Gly Asp Thr Ser Phe Gly Arg Gly Arg Lys Gly Gln
100 105 110

Lys Gly Glu Pro Gly Leu Val Pro Val Val Thr Gly Ile Arg Gly Arg
115 120 125

Pro Gly Pro Ala Gly Pro Pro Gly Ser Gln Gly Pro Arg Gly Asp Arg
130 135 140

Gly Pro Lys Gly Arg Pro Gly Pro Arg Gly Pro Gln Gly Ile Asp Gly
145 150 155 160

Glu Pro Gly Met Pro Gly Gln Pro Gly Ala Pro Gly Pro Pro Gly His
165 170 175

Pro Ser His Pro Gly Pro Asp Gly Met Ser Arg Pro Phe Ser Ala Gln
180 185 190

Met Ala Gly Leu Asp Glu Lys Ser Gly Leu Gly Ser Gln Val Gly Leu
195 200 205

Met Pro Gly Ser Val Gly Pro Val Gly Pro Arg Gly Pro Val Gly Leu
 210 215 220

Gln Gly Gln Gln Gly Gly Ala Gly Pro Ala Gly Pro Pro Gly Glu Pro
 225 230 235 240

Gly Glu Pro Gly Pro Met Gly Pro Ile Gly Ser Arg Gly Pro Glu Gly
 245 250 255

Pro Pro Gly Lys Pro Gly Glu Asp Gly Glu Pro Gly Arg Asn Gly Asn
 260 265 270

Thr Gly Glu Val Gly Phe Ser Gly Ser Pro Gly Ala Arg Gly Phe Pro
 275 280 285

Gly Ala Pro Gly Leu Pro Gly Leu Lys Gly His Arg Gly His Lys Gly
 290 295 300

Leu Glu Gly Pro Lys Gly Glu Ile Gly Ala Pro Gly Ala Lys Gly Glu
 305 310 315 320

Ala Gly Pro Thr Gly Pro Met Gly Ala Met Gly Pro Leu Gly Pro Arg
 325 330 335

Gly Met Pro Gly Glu Arg Gly Arg Leu Gly Pro Gln Gly Ala Pro Gly
 340 345 350

Lys Arg Gly Ala His Gly Met Pro Gly Lys Pro Gly Pro Met Gly Pro
 355 360 365

Leu Gly Ile Pro Gly Ser Ser Gly Phe Pro Gly Asn Pro Gly Met Lys
 370 375 380

Gly Glu Arg Gly Pro His Gly Ala Arg Gly Pro Glu Gly Pro Gln Gly
 385 390 395 400

Gln Arg Gly Glu Thr Gly Pro Pro Gly Pro Ala Gly Ser Gln Gly Leu
 405 410 415

Pro Gly Ala Val Gly Thr Asp Gly Thr Pro Gly Arg Lys Gly Ala Thr
 420 425 430

Gly Ser Ala Gly Thr Ser Gly Pro Pro Gly Leu Ala Gly Pro Pro Gly
 435 440 445

Ser Pro Gly Pro Gln Gly Ser Thr Gly Pro Gln Gly Ile Arg Gly Gln
 450 455 460

Ser Gly Asp Pro Gly Val Pro Gly Phe Lys Gly Glu Ala Gly Pro Lys
 465 470 475 480

Gly Glu Pro Gly Pro His Gly Ile Gln Gly Pro Ile Gly Pro Pro Gly-
 485 490 495

Glu Glu Gly Lys Arg Gly Pro Arg Gly Asp Pro Gly Thr Val Gly Pro
 500 505 510

Pro Gly Pro Met Gly Glu Arg Gly Ala Pro Gly Asn Arg Gly Phe Pro
 515 520 525

Gly Ser Asp Gly Leu Pro Gly Pro Lys Gly Ala Gln Gly Glu Arg Gly
 530 535 540

Pro Val Gly Ser Ser Gly Pro Lys Gly Gly Gln Gly Asp Pro Gly Arg
 545 550 555 560

Pro Gly Glu Pro Gly Leu Pro Gly Ala Arg Gly Leu Thr Gly Asn Pro
 565 570 575

Gly Val Gln Gly Pro Glu Gly Lys Leu Gly Pro Leu Gly Ala Pro Gly
 580 585 590

Glu Asp Gly Arg Pro Gly Pro Pro Gly Ser Ile Gly Ile Arg Gly Gln
 595 600 605

Pro Gly Ser Met Gly Val Pro Gly Pro Lys Gly Ser Ser Gly Asp Leu
 610 615 620

Gly Lys Pro Gly Glu Ala Gly Asn Ala Gly Val Pro Gly Gln Arg Gly
 625 630 635 640

Ala Pro Gly Lys Asp Gly Glu Val Gly Pro Ser Gly Pro Val Gly Pro
 645 650 655

Pro Gly Leu Ala Gly Glu Arg Gly Glu Ala Gly Pro Pro Gly Pro Thr
 660 665 670

Gly Phe Gln Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Glu Gly Gly
 675 680 685

Lys Ala Gly Asp Gln Gly Val Pro Gly Glu Pro Gly Ala Val Gly Pro
690 695 700

Leu Gly Pro Arg Gly Glu Arg Gly Asn Pro Gly Glu Arg Gly Glu Pro
705 710 715 720

Gly Ile Thr Gly Leu Pro Gly Glu Lys Gly Met Ala Gly Gly His Gly
725 730 735

Pro Asp Gly Pro Lys Gly Asn Pro Gly Pro Thr Gly Thr Ile Gly Asp
740 745 750

Thr Gly Pro Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ile Ala
755 760 765

Gly Thr Pro Gly Pro Lys Gly Asp Arg Gly Gly Ile Gly Glu Lys Gly
770 775 780

Ala Glu Gly Thr Ala Gly Asn Asp Gly Ala Arg Gly Leu Pro Gly Pro
785 790 795 800

Leu Gly Pro Pro Gly Pro Ala Gly Leu Leu Gly Ala Pro Gly Glu Pro
805 810 815

Gly Pro Arg Gly Leu Val Gly Pro Pro Gly Ser Arg Gly Asn Pro Gly
820 825 830

Ser Arg Gly Glu Asn Gly Pro Thr Gly Ala Val Gly Phe Ala Gly Pro
835 840 845

Gln Gly Ser Asp Gly Gln Pro Gly Val Lys Gly Glu Pro Gly Glu Pro
850 855 860

Gly Gln Lys Gly Asp Ala Gly Ser Pro Gly Pro Gln Gly Leu Ala Gly
865 870 875 880

Ser Pro Gly Pro His Gly Pro His Gly Val Pro Gly Leu Lys Gly Gly
885 890 895

Arg Gly Thr Gln Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ser Ala
900 905 910

Gly Arg Val Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly Pro Ala Gly
915 920 925

Pro Ala Gly Glu Pro Gly Lys Glu Gly Pro Pro Gly Leu Arg Gly Asp
 930 935 940

Pro Gly Ser His Gly Arg Val Gly Asp Arg Gly Pro Ala Gly Pro Pro
 945 950 955 960

Gly Ser Pro Gly Asp Lys Gly Asp Pro Gly Glu Asp Gly Gln Pro Gly
 965 970 975

Pro Asp Gly Pro Pro Gly Pro Ala Gly Thr Thr Gly Gln Arg Gly Ile
 980 985 990

Val Gly Met Pro Gly Gln Arg Gly Val Thr Gly Met Pro Gly Leu Pro
 995 1000 1005

Gly Pro Ala Gly Thr Pro Gly Lys Val Gly Pro Thr Gly Ala Thr
 1010 1015 1020

Gly Asp Lys Gly Pro Pro Gly Pro Val Gly Pro Pro Gly Ser Asn
 1025 1030 1035

Gly Pro Val Gly Glu Pro Gly Pro Glu Gly Pro Ala Gly Asn Asp
 1040 1045 1050

Gly Thr Pro Gly Arg Asp Gly Ala Val Gly Glu Arg Gly Asp Arg
 1055 1060 1065

Gly Asp Pro Gly Pro Ala Gly Leu Pro Gly Ser Gln Gly Ala Pro
 1070 1075 1080

Gly Thr Pro Gly Pro Val Gly Ala Pro Gly Asp Ala Gly Gln Arg
 1085 1090 1095

Gly Glu Pro Gly Ser Arg Gly Pro Val Gly Pro Pro Gly Arg Ala
 1100 1105 1110

Gly Lys Arg Gly Leu Pro Gly Pro Gln Gly Pro Arg Gly Asp Lys
 1115 1120 1125

Gly Asp Asn Gly Asp Arg Gly Asp Arg Gly Gln Lys Gly His Arg
 1130 1135 1140

Gly Phe Thr Gly Leu Gln Gly Leu Pro Gly Pro Pro Gly Pro Asn
 1145 1150 1155

Gly Glu Gln Gly Ser Ala Gly Ile Pro Gly Pro Phe Gly Pro Arg
 1160 1165 1170

Gly Pro Pro Gly Pro Val Gly Ser Ser Gly Lys Glu Gly Asn Pro
 1175 1180 1185

Gly Pro Leu Gly Pro Ile Gly Pro Pro Gly Val Arg Gly Ser Val
 1190 1195 1200

Gly Glu Ala Gly Pro Glu Gly Pro Pro Gly Glu Pro Gly Pro Pro
 1205 1210 1215

Gly Pro Pro Gly Pro Pro Gly His Leu Thr Ala Ala Leu Gly Asp
 1220 1225 1230

Ile Met Gly His Tyr Asp Glu Asn Met Pro Asp Pro Leu Pro Glu
 1235 1240 1245

Phe Thr Glu Asp Gln Ala Ala Pro Asp Asp Thr Asn Lys Thr Asp
 1250 1255 1260

Pro Gly Ile His Val Thr Leu Lys Ser Leu Ser Ser Gln Ile Glu
 1265 1270 1275

Thr Met Arg Ser Pro Asp Gly Ser Lys Lys His Pro Ala Arg Thr
 1280 1285 1290

Cys Asp Asp Leu Lys Leu Cys His Pro Thr Lys Gln Ser Gly Glu
 1295 1300 1305

Tyr Trp Ile Asp Pro Asn Gln Gly Ser Ala Glu Asp Ala Ile Lys
 1310 1315 1320

Val Tyr Cys Asn Met Glu Thr Gly Glu Thr Cys Ile Ser Ala Asn
 1325 1330 1335

Pro Ala Ser Val Pro Arg Lys Thr Trp Trp Ala Ser Lys Ser Pro
 1340 1345 1350

Asp Asn Lys Pro Val Trp Tyr Gly Leu Asp Met Asn Arg Gly Ser
 1355 1360 1365

Gln Phe Thr Tyr Gly Asp Tyr Gln Ser Pro Asn Thr Ala Ile Thr
 1370 1375 1380

Gln Met Thr Phe Phe Arg Leu Leu Ser Lys Glu Ala Ser Gln Asn
1385 1390 1395

Leu Thr Tyr Ile Cys Arg Asn Thr Val Gly Tyr Met Asp Asp Gln
1400 1405 1410

Ala Lys Asn Leu Lys Lys Ala Val Val Leu Lys Gly Ser Asn Asp
1415 1420 1425

Leu Glu Ile Lys Gly Glu Gly Asn Ile Arg Phe Arg Tyr Thr Val
1430 1435 1440

Leu Gln Asp Thr Cys Ser Lys Arg Asn Gly Asn Val Gly Lys Thr
1445 1450 1455

Ile Phe Glu Tyr Arg Thr Gln Asn Val Ala Arg Leu Pro Ile Ile
1460 1465 1470

Asp Val Gly Pro Val Asp Ile Gly Asn Ala Asp Gln Glu Phe Gly
1475 1480 1485

Leu Asp Ile Gly Pro Val Cys Phe Met
1490 1495

<210> 24

<211> 420

<212> PRT

<213> Cervus elaphus

<400> 24

Pro Gly Ala Pro Gly Ala Pro Gly Ala Pro Gly Pro Val Gly Pro Ala
1 5 10 15

Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro Ala Gly Pro Ala Gly
20 25 30

Pro Ile Gly Pro Val Gly Ala Arg Gly Pro Ala Gly Pro Gln Gly Pro
35 40 45

Arg Gly Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg Gly Ile Lys
50 55 60

Gly His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly Pro Pro Gly
65 70 75 80

Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro Ala Gly Pro
85 90 95

Arg Gly Pro Pro Gly Ser Ala Gly Thr Pro Gly Lys Asp Gly Leu Asn
 100 105 110

Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly Arg Thr Gly
 115 120 125

Asp Ala Gly Pro Ala Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro
 130 135 140

Pro Gly Pro Pro Ser Gly Gly Tyr Asp Leu Ser Phe Leu Pro Gln Pro
 145 150 155 160

Pro Gln Glu Lys Ala His Asp Gly Gly Arg Tyr Tyr Arg Ala Asp Asp
 165 170 175

Ala Asn Val Val Arg Asp Arg Asp Leu Glu Val Asp Thr Thr Leu Lys
 180 185 190

Ser Leu Ser Gln Gln Ile Glu Asn Ile Arg Ser Pro Glu Gly Ser Arg
 195 200 205

Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu Lys Met Cys His Ser Asp
 210 215 220

Trp Lys Ser Gly Glu Tyr Trp Ile Asp Pro Asn Gln Gly Cys Asn Leu
 225 230 235 240

Asp Ala Ile Lys Val Phe Cys Asn Met Glu Thr Gly Glu Thr Cys Val
 245 250 255

Tyr Pro Thr Gln Pro Ile Val Ala Gln Lys Asn Trp Tyr Ile Ser Lys
 260 265 270

Asn Pro Lys Asp Lys Arg His Val Trp Tyr Gly Glu Ser Met Thr Gly
 275 280 285

Gly Phe Gln Phe Glu Tyr Gly Gly Gln Gly Ser Asp Pro Ala Asp Val
 290 295 300

Ala Ile Gln Leu Thr Phe Leu Arg Leu Met Ser Thr Glu Ala Ser Gln
 305 310 315 320

Asn Ile Thr Tyr His Cys Lys Asn Ser Val Ala Tyr Met Asp Gln Gln
 325 330 335

Thr Gly Asn Leu Lys Lys Ala Leu Leu Leu Gln Gly Ser Asn Glu Ile
 340 345 350
 Glu Ile Arg Ala Glu Gly Asn Ser Arg Phe Thr Tyr Ser Val Thr Tyr
 355 360 365
 Asp Gly Cys Thr Ser His Thr Gly Ala Trp Gly Lys Thr Val Ile Glu
 370 375 380
 Tyr Lys Thr Thr Lys Thr Ser Arg Leu Pro Ile Ile Asp Val Ala Pro
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 Leu Asp Val Gly Ala Pro Asp Gln Glu Phe Gly Phe Asp Val Gly Pro
 405 410 415
 Val Cys Phe Leu
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 Asp Arg Asp Val Trp Lys Pro Glu Pro Cys Arg Ile Cys Val Cys Asp
 50 55 60
 Asn Gly Lys Val Leu Cys Asp Asp Val Ile Cys Asp Glu Thr Lys Asn
 65 70 75 80
 Cys Pro Gly Ala Glu Val Pro Glu Gly Glu Cys Cys Pro Val Cys Pro
 85 90 95
 Asp Gly Ser Glu Ser Pro Thr Asp Gln Glu Thr Thr Gly Val Glu Gly
 100 105 110
 Asp Thr Gly Pro Arg Gly Pro Arg Gly Pro Ala Gly Pro Pro Gly Arg
 115 120 125

Asp Gly Ile Pro Gly Gln Pro Gly Leu Pro Gly Pro Pro Gly Pro Pro
 130 135 140

Gly Pro Pro Gly Pro Pro Gly Leu Gly Gly Asn Phe Ala Pro Gln Leu
 145 150 155 160

Ser Tyr Gly Tyr Asp Glu Lys Ser Thr Gly Gly Ile Ser Val Pro Gly
 165 170 175

Pro Met Gly Pro Ser Gly Pro Arg Gly Leu Pro Gly Pro Pro Gly Ala
 180 185 190

Pro Gly Pro Gln Gly Phe Gln Gly Pro Pro Gly Glu Pro Gly Glu Pro
 195 200 205

Gly Ala Ser Gly Pro Met Gly Pro Arg Gly Pro Pro Gly Pro Pro Gly
 210 215 220

Lys Asn Gly Asp Asp Gly Glu Ala Gly Lys Pro Gly Arg Pro Gly Glu
 225 230 235 240

Arg Gly Pro Pro Gly Pro Gln Gly Ala Arg Gly Leu Pro Gly Thr Ala
 245 250 255

Gly Leu Pro Gly Met Lys Gly His Arg Gly Phe Ser Gly Leu Asp Gly
 260 265 270

Ala Lys Gly Asp Ala Gly Pro Ala Gly Pro Lys Gly Glu Pro Gly Ser
 275 280 285

Pro Gly Glu Asn Gly Ala Pro Gly Gln Met Gly Pro Arg Gly Leu Pro
 290 295 300

Gly Glu Arg Gly Arg Pro Gly Ala Pro Gly Pro Ala Gly Ala Arg Gly
 305 310 315 320

Asn Asp Gly Ala Thr Gly Ala Ala Gly Pro Pro Gly Pro Thr Gly Pro
 325 330 335

Ala Gly Pro Pro Gly Phe Pro Gly Ala Val Gly Ala Lys Gly Glu Ala
 340 345 350

Gly Pro Gln Gly Pro Arg Gly Ser Glu Gly Pro Gln Gly Val Arg Gly
 355 360 365

Glu Pro Gly Pro Pro Gly Pro Ala Gly Ala Ala Gly Pro Ala Gly Asn
 370 375 380

Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly Ala Asn Gly Ala Pro
 385 390 395 400

Gly Ile Ala Gly Ala Pro Gly Phe Pro Gly Ala Arg Gly Pro Ser Gly-
 405 410 415

Pro Gln Gly Pro Gly Gly Pro Pro Gly Pro Lys Gly Asn Ser Gly Glu
 420 425 430

Pro Gly Ala Pro Gly Ser Lys Gly Asp Thr Gly Ala Lys Gly Glu Pro
 435 440 445

Gly Pro Val Gly Val Gln Gly Pro Pro Gly Pro Ala Gly Glu Glu Gly
 450 455 460

Lys Arg Gly Ala Arg Gly Glu Pro Gly Pro Thr Gly Leu Pro Gly Pro
 465 470 475 480

Pro Gly Glu Arg Gly Gly Pro Gly Ser Arg Gly Phe Pro Gly Ala Asp
 485 490 495

Gly Val Ala Gly Pro Lys Gly Pro Ala Gly Glu Arg Gly Ser Pro Gly
 500 505 510

Pro Ala Gly Pro Lys Gly Ser Pro Gly Glu Ala Gly Arg Pro Gly Glu
 515 520 525

Ala Gly Leu Pro Gly Ala Lys Gly Leu Thr Gly Ser Pro Gly Ser Pro
 530 535 540

Gly Pro Asp Gly Lys Thr Gly Pro Pro Gly Pro Ala Gly Gln Asp Gly
 545 550 555 560

Arg Pro Gly Pro Pro Gly Pro Pro Gly Ala Arg Gly Gln Ala Gly Val
 565 570 575

Met Gly Phe Pro Gly Pro Lys Gly Ala Ala Gly Glu Pro Gly Lys Ala
 580 585 590

Gly Glu Arg Gly Val Pro Gly Pro Pro Gly Ala Val Gly Pro Ala Gly
 595 600 605

Lys Asp Gly Glu Ala Gly Ala Gln Gly Pro Pro Gly Pro Ala Gly Pro
610 615 620

Ala Gly Glu Arg Gly Glu Gln Gly Pro Ala Gly Ser Pro Gly Phe Gln
625 630 635 640

Gly Leu Pro Gly Pro Ala Gly Pro Pro Gly Glu Ala Gly Lys Pro Gly
645 650 655

Glu Gln Gly Val Pro Gly Asp Leu Gly Ala Pro Gly Pro Ser Gly Ala
660 665 670

Arg Gly Glu Arg Gly Phe Pro Gly Glu Arg Gly Val Gln Gly Pro Pro
675 680 685

Gly Pro Ala Gly Pro Arg Gly Ala Asn Gly Ala Pro Gly Asn Asp Gly
690 695 700

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705 710 715 720

Pro Gly Leu Gln Gly Met Pro Gly Glu Arg Gly Ala Ala Gly Leu Pro
725 730 735

Gly Pro Lys Gly Asp Arg Gly Asp Ala Gly Pro Lys Gly Ala Asp Gly
740 745 750

Ser Pro Gly Lys Asp Gly Val Arg Gly Leu Thr Gly Pro Ile Gly Pro
755 760 765

Pro Gly Pro Ala Gly Ala Pro Gly Asp Lys Gly Glu Ser Gly Pro Ser
770 775 780

Gly Pro Ala Gly Pro Thr Gly Ala Arg Gly Ala Pro Gly Asp Arg Gly
785 790 795 800

Glu Pro Gly Pro Pro Gly Pro Ala Gly Phe Ala Gly Pro Pro Gly Ala
805 810 815

Asp Gly Gln Pro Gly Ala Lys Gly Glu Pro Gly Asp Ala Gly Ala Lys
820 825 830

Gly Asp Ala Gly Pro Pro Gly Pro Ala Gly Pro Ala Gly Pro Pro Gly
835 840 845

Pro Ile Gly Asn Val Gly Ala Pro Gly Ala Lys Gly Ala Arg Gly Ser
850 855 860

Ala Gly Pro Pro Gly Ala Thr Gly Phe Pro Gly Ala Ala Gly Arg Val
865 870 875 880

Gly Pro Pro Gly Pro Ser Gly Asn Ala Gly Pro Pro Gly Pro Pro Gly-
885 890 895

Pro Ala Gly Lys Glu Gly Gly Lys Gly Pro Arg Gly Glu Thr Gly Pro
900 905 910

Ala Gly Arg Pro Gly Glu Val Gly Pro Pro Gly Pro Pro Gly Pro Ala
915 920 925

Gly Glu Lys Gly Ser Pro Gly Ala Asp Gly Pro Ala Gly Ala Pro Gly
930 935 940

Thr Pro Gly Pro Gln Gly Ile Ala Gly Gln Arg Gly Val Val Gly Leu
945 950 955 960

Pro Gly Gln Arg Gly Glu Arg Gly Phe Pro Gly Leu Pro Gly Pro Ser
965 970 975

Gly Glu Pro Gly Lys Gln Gly Pro Ser Gly Ala Ser Gly Glu Arg Gly
980 985 990

Pro Pro Gly Pro Met Gly Pro Pro Gly Leu Ala Gly Pro Pro Gly Glu
995 1000 1005

Ser Gly Arg Glu Gly Ala Pro Gly Ala Glu Gly Ser Pro Gly Arg
1010 1015 1020

Asp Gly Ser Pro Gly Ala Lys Gly Asp Arg Gly Glu Thr Gly Pro
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Ala Gly Pro Pro Gly Ala Pro Gly Ala Pro Gly Ala Pro Gly Pro
1040 1045 1050

Val Gly Pro Ala Gly Lys Ser Gly Asp Arg Gly Glu Thr Gly Pro
1055 1060 1065

Ala Gly Pro Ala Gly Pro Val Gly Pro Val Gly Ala Arg Gly Pro
1070 1075 1080

Ala Gly Pro Gln Gly Pro Arg Gly Asp Lys Gly Glu Thr Gly Glu
1085 1090 1095

Gln Gly Asp Arg Gly Ile Lys Gly His Arg Gly Phe Ser Gly Leu
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Gln Gly Pro Pro Gly Pro Pro Gly Ser Pro Gly Glu Gln Gly Pro
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Ser Gly Ala Ser Gly Pro Ala Gly Pro Arg Gly Pro Pro Gly Ser
1130 1135 1140

Ala Gly Ala Pro Gly Lys Asp Gly Leu Asn Gly Leu Pro Gly Pro
1145 1150 1155

Ile Gly Pro Pro Gly Pro Arg Gly Arg Thr Gly Asp Ala Gly Pro
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Val Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro
1175 1180 1185

Pro Ser Ala Gly Phe Asp Phe Ser Phe Leu Pro Gln Pro Pro Gln
1190 1195 1200

Glu Lys Ala His Asp Gly Gly Arg Tyr Tyr Arg Ala Asp Asp Ala
1205 1210 1215

Asn Val Val Arg Asp Arg Asp Leu Glu Val Asp Thr Thr Leu Lys
1220 1225 1230

Ser Leu Ser Gln Gln Ile Glu Asn Ile Arg Ser Pro Glu Gly Ser
1235 1240 1245

Arg Lys Asn Pro Ala Arg Thr Cys Arg Asp Leu Lys Met Cys His
1250 1255 1260

Ser Asp Trp Lys Ser Gly Glu Tyr Trp Ile Asp Pro Asn Gln Gly
1265 1270 1275

Cys Asn Leu Asp Ala Ile Lys Val Phe Cys Asn Met Glu Thr Gly
1280 1285 1290

Glu Thr Cys Val Tyr Pro Thr Gln Pro Ser Val Ala Gln Lys Asn
1295 1300 1305

Trp Tyr Ile Ser Lys Asn Pro Lys Asp Lys Arg His Val Trp Phe
 1310 1315 1320
 Gly Glu Ser Met Thr Asp Gly Phe Gln Phe Glu Tyr Gly Gly Gln
 1325 1330 1335
 Gly Ser Asp Pro Ala Asp Val Ala Ile Gln Leu Thr Phe Leu Arg -
 1340 1345 1350
 Leu Met Ser Thr Glu Ala Ser Gln Asn Ile Thr Tyr His Cys Lys
 1355 1360 1365
 Asn Ser Val Ala Tyr Met Asp Gln Gln Thr Gly Asn Leu Lys Lys
 1370 1375 1380
 Ala Leu Leu Leu Lys Gly Ser Asn Glu Ile Glu Ile Arg Ala Glu
 1385 1390 1395
 Gly Asn Ser Arg Phe Thr Tyr Ser Val Thr Val Asp Gly Cys Thr
 1400 1405 1410
 Ser His Thr Gly Ala Trp Gly Lys Thr Val Ile Glu Tyr Lys Thr
 1415 1420 1425
 Thr Lys Thr Ser Arg Leu Pro Ile Ile Asp Val Ala Pro Leu Asp
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 Val Gly Ala Pro Asp Gln Glu Phe Gly Phe Asp Val Gly Pro Val
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 Cys Phe Leu
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 His Asn Gly Leu Arg Val Pro Asn Gly Glu Thr Trp Lys Pro Glu Val
 35 40 45

Cys Leu Ile Cys Ile Cys His Asn Gly Thr Ala Val Cys Asp Asp Val
50 55 60

Gln Cys Asn Glu Glu Leu Asp Cys Pro Asn Pro Gln Arg Arg Glu Gly
65 70 75 80

Gly Cys Cys Ala Phe Cys Pro Glu Glu Tyr Val Ser Pro Asn Ser Glu
85 90 95

Asp Val Gly Val Glu Gly Pro Lys Gly Gly Pro Gly Pro Gln Gly Pro
100 105 110

Arg Gly Pro Val Gly Pro Pro Gly Arg Asp Gly Ile Pro Gly Gln Pro
115 120 125

Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly
130 135 140

Leu Gly Gly Asn Phe Ala Ser Gln Met Ser Tyr Gly Tyr Asp Glu Lys
145 150 155 160

Ser Ala Gly Val Ser Val Pro Gly Pro Met Gly Pro Ser Gly Pro Arg
165 170 175

Gly Leu Pro Gly Pro Pro Gly Ala Pro Gly Pro Gln Gly Phe Gln Gly
180 185 190

Pro Pro Gly Glu Pro Gly Glu Pro Gly Gly Ser Gly Pro Met Gly Pro
195 200 205

Arg Gly Pro Pro Gly Pro Pro Gly Lys Asn Gly Asp Asp Gly Glu Ala
210 215 220

Gly Lys Pro Gly Arg Pro Gly Glu Arg Gly Pro Pro Gly Pro Gln Gly
225 230 235 240

Ala Arg Gly Leu Pro Gly Thr Ala Gly Leu Pro Gly Met Lys Gly His
245 250 255

Arg Gly Phe Ser Gly Leu Asp Gly Ala Lys Gly Asp Ala Gly Pro Ala
260 265 270

Gly Pro Lys Gly Glu Pro Gly Ser Pro Gly Glu Asn Gly Ala Pro Gly
275 280 285

Gln Met Gly Pro Arg Gly Leu Pro Gly Glu Arg Gly Arg Pro Gly Pro
 290 295 300

Pro Gly Thr Ala Gly Ala Arg Gly Asn Asp Gly Ala Val Gly Ala Ala
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Gly Pro Pro Gly Pro Thr Gly Pro Thr Gly Pro Pro Gly Phe Pro Gly-
 325 330 335

Ala Val Gly Ala Lys Gly Glu Ala Gly Pro Gln Gly Ala Arg Gly Ser
 340 345 350

Glu Gly Pro Gln Gly Val Arg Gly Glu Pro Gly Pro Pro Gly Pro Ala
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Gly Ala Ala Gly Pro Ala Gly Asn Pro Gly Ala Asp Gly Gln Pro Gly
 370 375 380

Ala Lys Gly Ala Asn Gly Ala Pro Gly Ile Ala Gly Ala Pro Gly Phe
 385 390 395 400

Pro Gly Ala Arg Gly Pro Ser Gly Pro Gln Gly Pro Ser Gly Pro Pro
 405 410 415

Gly Pro Lys Gly Asn Ser Gly Glu Pro Gly Ala Pro Gly Asn Lys Gly
 420 425 430

Asp Thr Gly Ala Lys Gly Glu Pro Gly Ala Thr Gly Val Gln Gly Pro
 435 440 445

Pro Gly Pro Ala Gly Glu Glu Gly Lys Arg Gly Ala Arg Gly Glu Pro
 450 455 460

Gly Pro Ser Gly Leu Pro Gly Pro Pro Gly Glu Arg Gly Gly Pro Gly
 465 470 475 480

Ser Arg Gly Phe Pro Gly Ala Asp Gly Val Ala Gly Pro Lys Gly Pro
 485 490 495

Ser Gly Glu Arg Gly Ala Pro Gly Pro Ala Gly Pro Lys Gly Ser Pro
 500 505 510

Gly Glu Ala Gly Arg Pro Gly Glu Ala Gly Leu Pro Gly Ala Lys Gly
 515 520 525

Leu Thr Gly Ser Pro Gly Ser Pro Gly Pro Asp Gly Lys Thr Gly Pro
530 535 540

Pro Gly Pro Ala Gly Gln Asp Gly Arg Pro Gly Pro Ala Gly Pro Pro
545 550 555 560

Gly Ala Arg Gly Gln Ala Gly Val Met Gly Phe Pro Gly Pro Lys Gly
565 570 575

Thr Ala Gly Glu Pro Gly Lys Ala Gly Glu Arg Gly Leu Pro Gly Pro
580 585 590

Pro Gly Ala Val Gly Pro Ala Gly Lys Asp Gly Glu Ala Gly Ala Gln
595 600 605

Gly Ala Pro Gly Pro Ala Gly Pro Ala Gly Glu Arg Gly Glu Gln Gly
610 615 620

Pro Ala Gly Ser Pro Gly Phe Gln Gly Leu Pro Gly Pro Ala Gly Pro
625 630 635 640

Pro Gly Glu Ala Gly Lys Pro Gly Glu Gln Gly Val Pro Gly Asp Leu
645 650 655

Gly Ala Pro Gly Pro Ser Gly Ala Arg Gly Glu Arg Gly Phe Pro Gly
660 665 670

Glu Arg Gly Val Gln Gly Pro Pro Gly Pro Ala Gly Pro Arg Gly Asn
675 680 685

Asn Gly Ala Pro Gly Asn Asp Gly Ala Lys Gly Asp Thr Gly Ala Pro
690 695 700

Gly Ala Pro Gly Ser Gln Gly Ala Pro Gly Leu Gln Gly Met Pro Gly
705 710 715 720

Glu Arg Gly Ala Ala Gly Leu Pro Gly Pro Lys Gly Asp Arg Gly Asp
725 730 735

Ala Gly Pro Lys Gly Ala Asp Gly Ser Pro Gly Lys Asp Gly Ala Arg
740 745 750

Gly Leu Thr Gly Pro Ile Gly Pro Pro Gly Pro Ala Gly Ala Pro Gly
755 760 765

Asp Lys Gly Glu Ala Gly Pro Ser Gly Pro Pro Gly Pro Thr Gly Ala
770 775 780

Arg Gly Ala Pro Gly Asp Arg Gly Glu Ala Gly Pro Pro Gly Pro Ala
785 790 795 800

Gly Phe Ala Gly Pro Pro Gly Ala Asp Gly Gln Pro Gly Ala Lys Gly-
805 810 815

Glu Pro Gly Asp Thr Gly Val Lys Gly Asp Ala Gly Pro Pro Gly Pro
820 825 830

Ala Gly Pro Ala Gly Pro Pro Gly Pro Ile Gly Asn Val Gly Ala Pro
835 840 845

Gly Pro Lys Gly Pro Arg Gly Ala Ala Gly Pro Pro Gly Ala Thr Gly
850 855 860

Phe Pro Gly Ala Ala Gly Arg Val Gly Pro Pro Gly Pro Ser Gly Asn
865 870 875 880

Ala Gly Pro Pro Gly Pro Pro Gly Pro Val Gly Lys Glu Gly Gly Lys
885 890 895

Gly Pro Arg Gly Glu Thr Gly Pro Ala Gly Arg Pro Gly Glu Val Gly
900 905 910

Pro Pro Gly Pro Pro Gly Pro Ala Gly Glu Lys Gly Ser Pro Gly Ala
915 920 925

Asp Gly Pro Ala Gly Ser Pro Gly Thr Pro Gly Pro Gln Gly Ile Ala
930 935 940

Gly Gln Arg Gly Val Val Gly Leu Pro Gly Gln Arg Gly Glu Arg Gly
945 950 955 960

Phe Pro Gly Leu Pro Gly Pro Ser Gly Glu Pro Gly Lys Gln Gly Pro
965 970 975

Ser Gly Ser Ser Gly Glu Arg Gly Pro Pro Gly Pro Met Gly Pro Pro
980 985 990

Gly Leu Ala Gly Pro Pro Gly Glu Ser Gly Arg Glu Gly Ser Pro Gly
995 1000 1005

Ala Glu Gly Ser Pro Gly Arg Asp Gly Ala Pro Gly Ala Lys Gly
1010 1015 1020

Asp Arg Gly Glu Thr Gly Pro Ala Gly Pro Pro Gly Ala Pro Gly
1025 1030 1035

Ala Pro Gly Ala Pro Gly Pro Val Gly Pro Ala Gly Lys Asn Gly
1040 1045 1050

Asp Arg Gly Glu Thr Gly Pro Ala Gly Pro Ala Gly Pro Ile Gly
1055 1060 1065

Pro Ala Gly Ala Arg Gly Pro Ala Gly Pro Gln Gly Pro Arg Gly
1070 1075 1080

Asp Lys Gly Glu Thr Gly Glu Gln Gly Asp Arg Gly Ile Lys Gly
1085 1090 1095

His Arg Gly Phe Ser Gly Leu Gln Gly Pro Pro Gly Ser Pro Gly
1100 1105 1110

Ser Pro Gly Glu Gln Gly Pro Ser Gly Ala Ser Gly Pro Ala Gly
1115 1120 1125

Pro Arg Gly Pro Pro Gly Ser Ala Gly Ser Pro Gly Lys Asp Gly
1130 1135 1140

Leu Asn Gly Leu Pro Gly Pro Ile Gly Pro Pro Gly Pro Arg Gly
1145 1150 1155

Arg Thr Gly Asp Ser Gly Pro Ala Gly Pro Pro Gly Pro Pro Gly
1160 1165 1170

Pro Pro Gly Pro Pro Gly Pro Pro Ser Gly Gly Tyr Asp Phe Ser
1175 1180 1185

Phe Leu Pro Gln Pro Pro Gln Glu Lys Ser Gln Asp Gly Asp Arg
1190 1195 1200

Tyr Tyr Arg Ala Asp Asp Ala Asn Val Val Arg Asp Arg Asp Leu
1205 1210 1215

Ala Val Asp Ala Thr Leu Lys Ser Leu Ser Gln Gln Ile Glu Asn
1220 1225 1230

Ile Arg Ser Pro Glu Gly Ser Arg Lys Asn Pro Ala Arg Thr Cys
 1235 1240 1245
 Arg Asp Leu Lys Met Cys His Ser Asp Trp Lys Ser Gly Glu Tyr
 1250 1255 1260
 Trp Ile Asp Pro Asn Gln Gly Cys Asn Leu Asp Ala Ile Lys Val -
 1265 1270 1275
 Tyr Cys Asn Met Glu Thr Gly Gln Thr Cys Val Phe Pro Thr Gln
 1280 1285 1290
 Pro Ser Val Pro Gln Lys Asn Trp Tyr Ile Ser Pro Asn Pro Lys
 1295 1300 1305
 Glu Lys Lys His Val Trp Phe Gly Glu Ser Met Thr Asp Gly Phe
 1310 1315 1320
 Pro Phe Glu Tyr Gly Ser Glu Gly Ser Asp Pro Thr Asp Val Ala
 1325 1330 1335
 Ile Gln Leu Thr Phe Leu Arg Leu Met Ser Thr Glu Ala Ser Gln
 1340 1345 1350
 Asn Ile Thr Tyr His Cys Lys Asn Ser Val Ala Tyr Met Asp Gln
 1355 1360 1365
 Gln Thr Gly Asn Leu Lys Lys Ala Leu Leu Leu Gln Gly Ser Asn
 1370 1375 1380
 Glu Ile Glu Leu Arg Gly Glu Gly Asn Ser Arg Phe Thr Tyr Ser
 1385 1390 1395
 Arg Val Val Asp Gly Cys Thr Ser His Thr Gly Thr Trp Gly Lys
 1400 1405 1410
 Thr Val Ile Glu Tyr Lys Thr Thr Lys Thr Ser Arg Leu Pro Ile
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 Gly Leu Asp Ile Gly Pro Ala Cys Phe Val
 1445 1450

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 <213> Homo sapiens
 <400> 27

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Val Ser Glu Ala Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu
 20 25 30

Met Val Lys Val Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val
 35 40 45

Ala Val His Val Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe
 50 55 60

Ala Ser Gly Lys Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr
 65 70 75 80

Glu Glu Glu Phe Val Glu Gly Ile Tyr Lys Val Glu Ile Asp Thr Lys
 85 90 95

Ser Tyr Trp Lys Ala Leu Gly Ile Ser Pro Phe His Glu His Ala Glu
 100 105 110

Val Val Phe Thr Ala Asn Asp Ser Gly Pro Arg Arg Tyr Thr Ile Ala
 115 120 125

Ala Leu Leu Ser Pro Tyr Ser Tyr Ser Thr Thr Ala Val Val Thr Asn
 130 135 140

Pro Lys Glu
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 <211> 1426
 <212> DNA
 <213> Cervus elaphus

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 gaccccttgg cctcctctggc ccccccggtc ctggcattga catgtctgcc ttggtctggc 180
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 cccagaacat cacctaccac tgcaagaaca gcattgccta cctggacgaa gctgctggca 720
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 cccagcccag gcaagaggcc cccctgcagg tgccggggcg gggaactgtg tgtgtcctac 1320
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<210> 29

<211> 957

<212> DNA

<213> Cervus elaphus

<400> 29

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taagctgtcc atcgteccccg tgcgaagggg ctactggggg aacaagatcg gcaagcccca 540
 cacggttcct tgcaaggtga ctggcgcgtg tggctcctg ctggtgcgcc tcatccctgc 600
 cccagaggc actggcatcg tctccgcccc tgtgcccaag aagctgctga tgatggccgg 660
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 ggccactttt gatgccattt ccaagacctt cagttacctc actcctgacc tctggaaaga 780
 gacgggtgttc accaagtctc catatcagga atttactgac catcttgtga agaccacac 840
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 aattttacaa agagaataat aaagtgaatg aaaccggaaa aaaaaaaaaa aaaaaaa 957

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 <211> 532
 <212> DNA
 <213> Cervus elaphus

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 caccacactt ccggcggccc aaaacactgc ggctcaggag gcagcccaaa tatcctcgga 180
 agagcgcccc caggagaaac aaacttgacc actatgccat catcaaattc cccctacca 240
 ctgagtcagc catgaagaaa atagaagaca acaacacact ggtattcatt gtggatgtca 300
 aggccaacaa gcaccaaatt aaacaggctg tgaagaagct ctatgacatt gacgtggcta 360
 aggtcaatac tctgatcagg cctgatggag agaagaaggc atatgttcga ctggctcctg 420
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 taattccaaa tataagtttt cactatgtaa aaaaaaaaaa aaaaaaaaaa aa 532

<210> 31
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 <212> DNA
 <213> Cervus elaphus

<400> 31
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 gcggcgaagg aggagcccaa gaggagatcg gcgaggttgt cagcaaaacc ggctcctgca 180
 aaagtggaaa cgaagccaaa aaaggcggcg ggaaaggata aatcttcaga caaaaaagtg 240
 caaacaaaag ggaaaagagg agcaaaggga aaacaggcgg aagtggccaa ccaagagact 300
 aaagaagact tgcctgcaga aaatggagag actaaaaacg aggagagccc agcctctgat 360
 gaagcagaag agaaagaagc caagtctgat taataaccac acactcagtc ctgtcagtg 420

tccctgtttc ccttcttgta caatccagag gaatatTTTT atcaactatt ttgtaaattgc 480
 aagtttttta gtagctctag aaacattttt aaaaaggagg gaatcccacc tcatcccatt 540
 ttttaagtgt aaatgctttt ttttaagagg tgaaatcatt tgctgggttg gttatttttt 600
 ggtacaacca gaaaatagtg ggatattgga tatgggaggc tttgattgtc ttgggtgtca 660
 acttaacatt ccttagatgg ggggagcttt tatatcctat aatacaaaag catactaaat 720
 ggcagtttgg agtcagttgt gcatttaatg tcttgaacac tttaaattac ttctcttccc 780
 attttgtttt ggtagaatta tttcctacag caaaccactt tttgatcttg gctctcctgg 840
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 aaaaaaaaaa aaaaaaaaaa aaaa 1224

<210> 32
 <211> 1163
 <212> DNA
 <213> *Cervus elaphus*

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 gccattgtca ctttcatcac caaagtttta ctgggcccaga ggaaatgaag gaaggccaca 660
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 gatggaatta tgccctgttg gttccaatgc tgggactagc tgcttttcgc tggatttggt 180
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<212> DNA
<213> Cervus elaphus

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<212> DNA
<213> Cervus elaphus

```


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 gggctgctgg aggccgagct ggctgcgctc tgccctgccg cgctggcccc ctactacctg 180
 cgcgcaccca gcgtggcgct gcctaccgcc caggtatcga ccgaccccg gcatctctcg 240
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 <211> 588
 <212> DNA
 <213> Cervus elaphus

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 <221> misc_feature
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<223> N=unknown

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 acagacctcc taccgggac catcctggcc tggctcncaa gactggatcc tccccctcat 180
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 <212> DNA
 <213> Cervus elaphus

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 cccccaggc ccagttggtc cttcaggcaa agaaggaagc cctgggcgc ttgggcccac 180

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catggggcac tatgatgaga gcatgccaga ccacttccg gagtttactg aagatcaggc 360
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 <212> DNA
 <213> Cervus elaphus

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<213> Artificial sequence

<220>

<223> Oligonucleotide primer

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<210> 40
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<213> Artificial sequence

<220>

<223> Oligonucleotide primer

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<210> 41
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<223> X=L or I

<220>

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<222> (7)..(7)

<223> X=Q or K

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1 5 10

<210> 42
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<213> Dama dama

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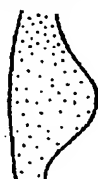
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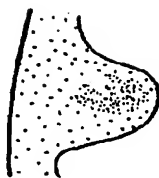
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1 5 +

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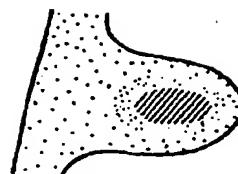
CHONDROCYTE DIFFERENTIATION



A



B



C



D



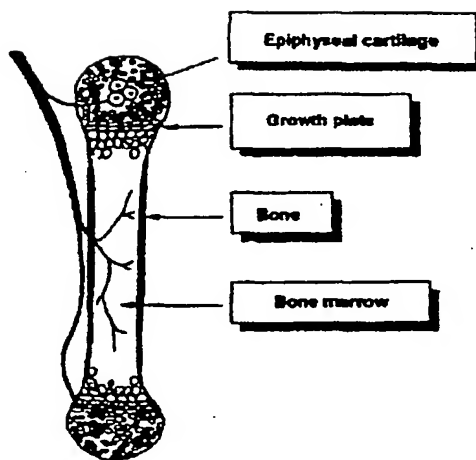
E



F



G



H

Figure 1

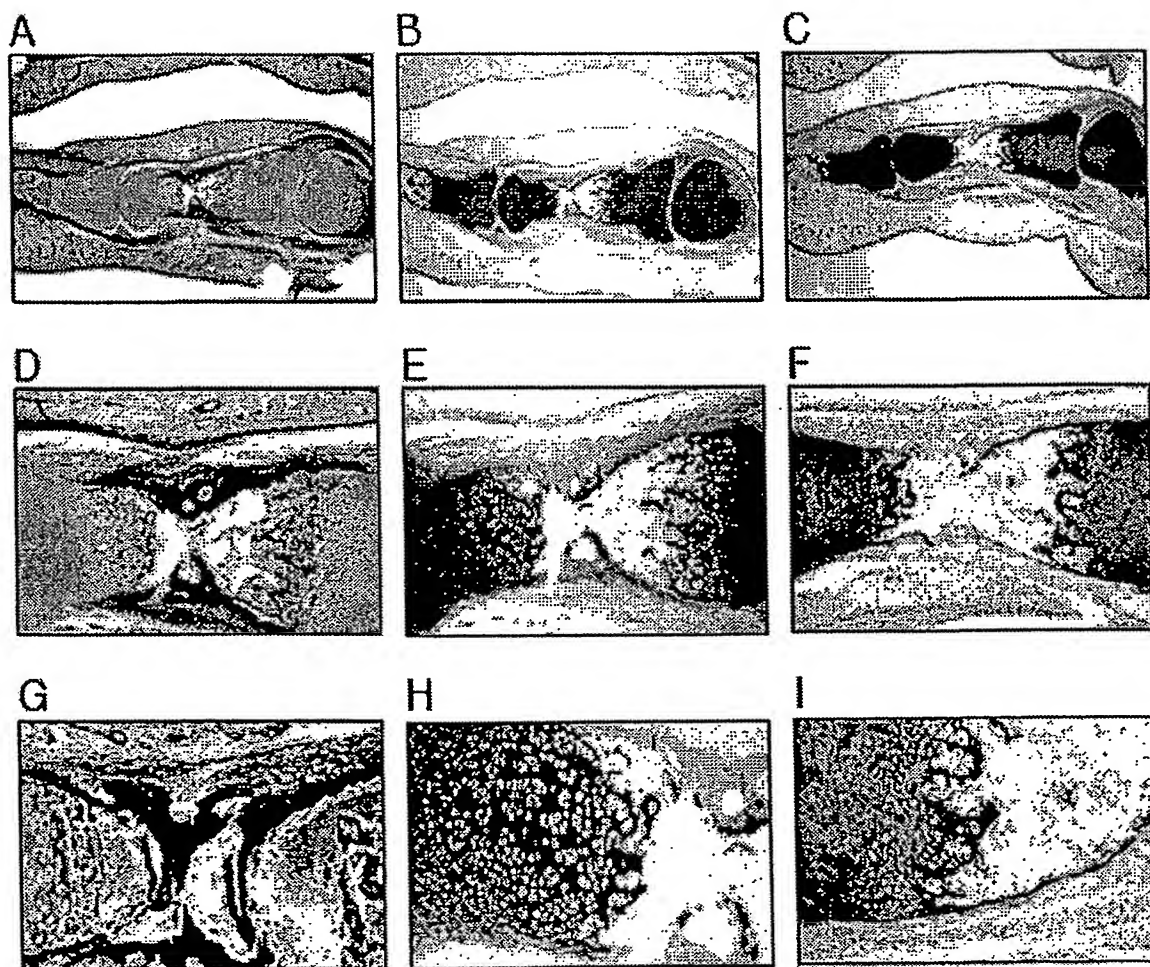
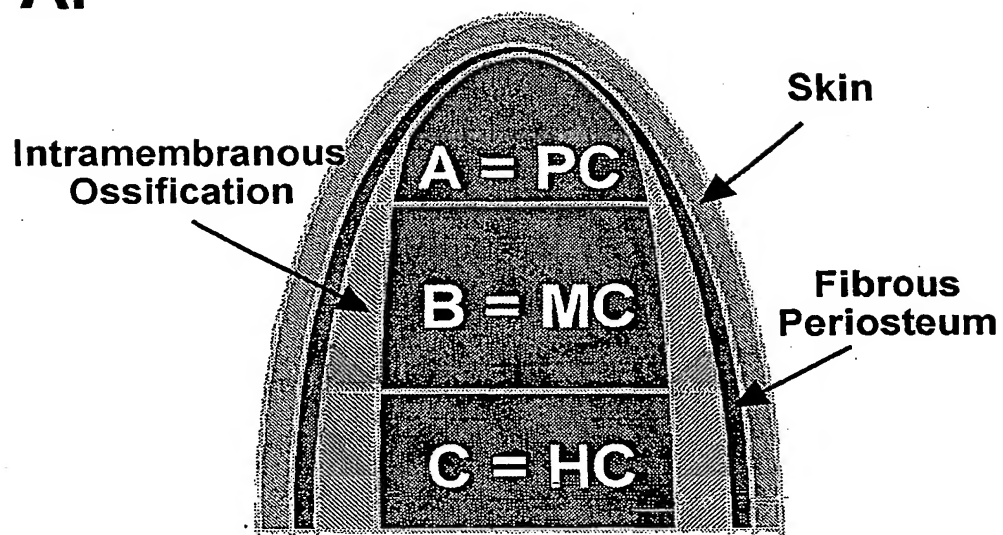


Figure 2

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A.



B.

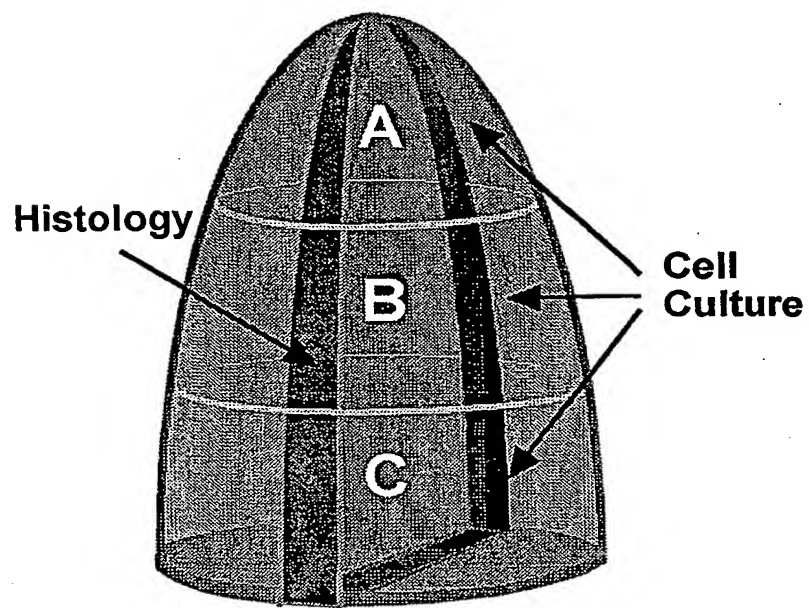
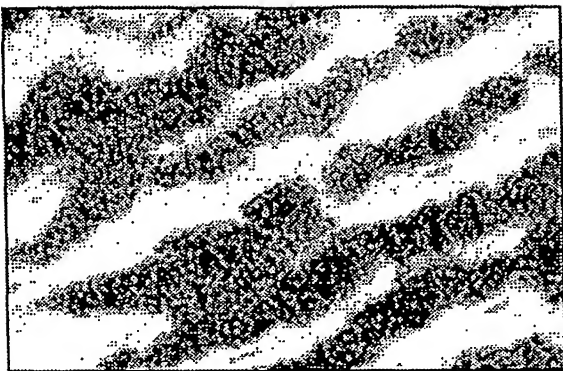


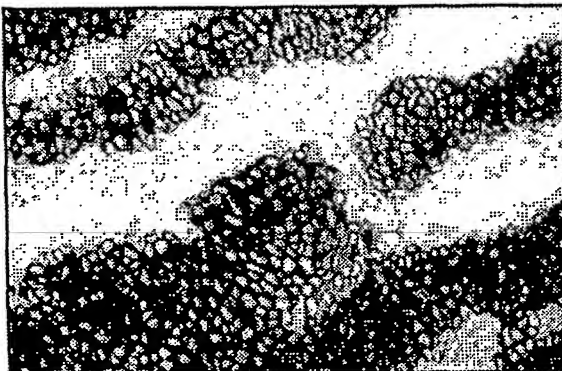
Figure 3

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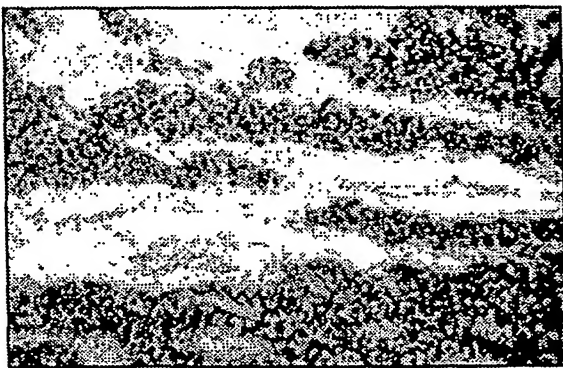
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B



C



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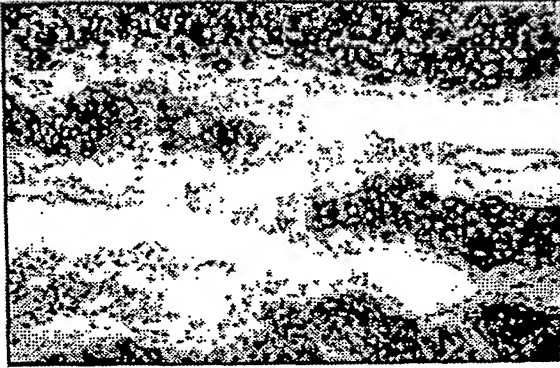


Figure 4

(i) DACC-2

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 101 TGGTCCTCCT GGAAACCCTG GACCCCTGG CCCTCCTGGC CCCCCGGTC
 151 CTGGCATTGA CATGTCTGCC TTTGCTGGCC TCGGCCAGAG AGAGAAGGGC
 201 CCCGACCCCC TGCAGTACAT GCGGGCCGAT GAGGCAGCCG GCAACCTGAG
 251 ACAGCATGAT CCCGAGGTGG ACGCCACACT CAAGTCCCTC AACAACCAGA
 301 TCGAGAGCCT CCGCAGCCCC GAGGGCTCAC GCAAGAACCC AGCTCGCACC
 351 TGCCGAGACC TGAAACTCTG CCACCCTGAG TGGAAGAGCG GAGACTACTG
 401 GATCGACCCC AACCAGGGCT GCACCCTGGA TGCCATGAAG GTTTTCTGCA
 451 ACATGGAGAC TGGCGAGACC TGCCTCTACC CCAACCCAGC CAGTGTTCCC
 501 AAGAAGAACT GGTGGAGCAG CAAGAGCAAG GACAAGAAAC ACATCTGGTT
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 951 CCTGTCTGCT TCTTGTA AAA ACCCGAACCC AGAACCAACA CAATCCATTG
 1001 CAAACCCAAA GGACCCAAGT ACTTTC CAAT CCCAGTCACT CTAGGACTCT
 1051 GCACTGAATG GCTGATCTGA CCTGACGCC ATTCATCCCA CCCGCTCACA
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 1151 AATAAAATCT CGGTGTTCTA TTTATTTATT GTCTTCCTGT AAGACCTTTG
 1201 GGTCAAGGCA GAGACAGGAA ACTAACTGGT GTGAGTCAAA TGCCCCCTGA
 1251 GTGACTGCCC CCCAGCCCAG GCAAGAGGCC CCCCTGCAGG TGCCGGGCGC
 1301 GGGAACGTG TGTGTCCTAC ACAATGGTGC TATTCTGTGT CAAACACCTC

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1351 TGTATTTTTT AAGACGTCAA TTGATATTAA AAACAAAAAA ATTATTGGAA

1401 AGTAAAAAAA AAAAAAAAAA AAAAAA

(ii) DACC-3

1 CCACCAAATG GCGGATGACG CCGGTGCTGC GGGAGGGCCC GGAGGCCCGG
 51 GGGGCCCTGG AATGGGAGGC CGCGGTGGCT TCCGCGGAGG CTTCCGTAGT
 101 GGCGTCCGGG GCCGGGGTCG TGGCCGCGGT CGGGGCCGGG GCAGAGGCCG
 151 CGGAGCTCGC GGAGGCAAGG CCGAGGACAA GGAGTGGCTC CCCGTTACCA
 201 AGCTGGGCCG CCTGGTCAAG GACATGAAGA TCAAGTCCCT GGAGGAGATC
 251 TACCTTTTCT CTCTGCCCAT CAAGGAGTCT GAGATTATTG ACTTTTTTCT
 301 GGGAGCGTCC CTCAAGGATG AAGTTTTGAA GATTATGCCG GTGCAAAAGC
 351 AGACCCGTGC TGGCCAGCGG ACCAGGTTCA AGGCATTTGT TGCCATCGGG
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 951 AAAAAAA

(iii) DACC-4

1 CGAAGGCGAA GAAGGAAGCC CCTGCCCTC CTAAAGCTGA AGCCAAAGCA
 51 AAGGCTTTGA AGGCCAAGAA AGCAGTGTTG AAAGGTGTCC ACAGCCACAA
 101 GAAAAAGAAG ATCCGGACGT CACCCACCTT CCGGCGGCCC AAAACACTGC
 151 GGCTCAGGAG GCAGCCCAA TATCCTCGGA AGAGCGCCCC CAGGAGAAAC

Figure 5 continues on the following page

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 251 CATGAAGAAA ATAGAAGACA ACAACACACT GGTATTCATT GTGGATGTCA
 301 AGGCCAACAA GCACCAAATT AACAGGCTG TGAAGAAGCT CTATGACATT
 351 GACGTGGCTA AGGTCAATAC TCTGATCAGG CCTGATGGAG AGAAGAAGGC
 401 ATATGTTCTGA CTGGCTCCTG ACTATGATGC TTTGGATGTT GCCAACAAAA
 451 TTGGGATCAT CTAACTGAG TCCAGCTGGC TAATTCCAAA TATAAGTTTT
 501 CACTATGTAA AAAAAAAAAA AAAAAAAAAA AA

(iv) DACC-5

1 GGCAGCGGTC AGGCTACTCA GCTTCGCGAA GGCTCTCGGC GCGCCGCGGC
 51 CCTCAGGCAC CCGGCTCTCG CCCGCCCGC CGCCACGATG CCAAGAGGA
 101 AGGTCAGCTC CGCCGAGGGG GCGGCGAAGG AGGAGCCCAA GAGGAGATCG
 151 GCGAGGTTGT CAGCAAAACC GGCTCCTGCA AAAGTGGAAG CGAAGCCAAA
 201 AAAGGCGGCG GGAAAGGATA AATCTTCAGA CAAAAAGTG CAAACAAAAG
 251 GGAAAAGAGG AGCAAAGGGA AACAGGCGG AAGTGGCCAA CCAAGAGACT
 301 AAAGAAGACT TGCCTGCAGA AAATGGAGAG ACTAAAAACG AGGAGAGCCC
 351 AGCCTCTGAT GAAGCAGAAG AGAAAGAAGC CAAGTCTGAT TAATAACCAC
 401 ACACTCAGTC CTGTCAGTGG TCCCTGTTTC CCTTCTTGTA CAATCCAGAG
 451 GAATATTTTT ATCAACTATT TTGTAAATGC AAGTTTTTTA GTAGCTCTAG
 501 AAACATTTTT AAAAAGGAGG GAATCCCACC TCATCCCATT TTTTAAGTGT
 551 AAATGCTTTT TTTTAAGAGG TGAAATCATT TGCTGGGTTG GTTATTTTTT
 601 GGTACAACCA GAAAATAGTG GGATATTGGA TATGGGAGGC TTTGATTGTC
 651 TTGGGTGTCA ACTTAACATT CCTTAGATGG GGGGAGCTTT TATATCCTAT
 701 AATACAAAAG CATACTAAAT GGCAGTTTGG AGTCAGTTGT GCATTTAATG
 751 TCTTGAACAC TTAAATTAC TTCTCTCCC ATTTTGTTTT GGTAGAATTA
 801 TTTCCTACAG CAAACCACTT TTTGATCTTG GCTCTCCTGG TCAGAATTTT
 851 GTGCACTATA CTATAACATC TTTGGTCGTG GTAGTCCAGT TTTCTAGTA
 901 ACTTGGTTAA TGTGCTGTGA ACGATTGACA GTTTGGGTAT GTAGTGTATA
 951 TGATATTAAA TTGTGAATCA GTGGGACTTA TGATGTAACA ACATATCAAT

Figure 5 continues on the following page

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1001 ATTGAAGAT ATTGGTACTT GATATCCTGT TAAGGAAAGT TGCTCCAAAT
 1051 TTTAAGCTGG AAAGTCACTG GAATAACTGT TAAGAATCAC AACTACATGA
 1101 TATTTTAGAT TTCTGGTACG TATGTGAAGA ATTGTGTACC AATTGAAATA
 1151 TCTGTGTAGT GATCCTCAAA ACAACCAATA AAATCTCCGT TATAAAAGAA
 1201 AAAAAAAAAA AAAAAAAAAA AAAA

(v) DACC-6

1 TCGGAACAGC TGGTCCGCCA TTTTCTCATT GAGACTGGGC CCAAAGGGGT
 51 GAAGATCAAG GGCTGTCCCA GCGAGCCCTA CTTTGGCAGC CTATCAGCCC
 101 TGGTCTCCCA GCACTCCATC TCCCCACTGT CCCTGCCCTG CTGCCTGCGC
 151 ATTCCCAGCA AAGATCCTCT GGAGGAGGTC CCAGAGGCCC CAGTGCCCAG
 201 CAACATGAGT ACGGCAGCAG ACCTCCTGCG TCAGGGCGCC GCCTGCAGTG
 251 TGCTCTACCT GACCTCAGTG GAGACGGAGT CGCTGACGGG CCCCCAAGCG
 301 GTGGCACGGG CCAGCTCCGC GGCTCTGAGC TGCAGCCCCC GCCCCACGCC
 351 AGCCGTTGTC CACTTCAAGG TCTCAGCCCA GGGCATCACA CTCACAGACA
 401 ACCAAAGGAA GCTCTTCTTT CGCCGCCATT ATCCAGTGAA CAGCATCACC
 451 TTCTCCAGCA CTGACCCTCA GGACCGGAGA TGGACCAACT CCGACGGGAC
 501 CACCTCCAAG ATCTTTGGTT TCGTGGCCAA GAAGCCGGGA AGCCCTTGGG
 551 AGAATGTGTG TCACCTCTTT GCAGAGCTTG ACCCAGATCA GCCTGCAGGC
 601 GCCATTGTCA CCTTCATCAC CAAAGTTTTA CTGGGCCAGA GGAAATGAAG
 651 GAAGGCCACA AGCTCCAAGC CCGCGTCAAC ACTGTGCCCC TCTCAGCACC
 701 ACACAGCCCT CACTTCCCCT GGCCTGGACC CAGGAGACCC AGGAGCCGCC
 751 TCTCCCCTAG GAATGGGGAG CAGACACACC GGCCTGCAAC ACTGCTCTCC
 801 TTCCCCGCCC CCAGCCTGCT AAGCAAGTGG ATGGGCCCAT GAGATGACCT
 851 TGCATGTGAG CAGAGGGCAG AGACGGGTGT GTGAGGGTGA GGTGGTGGAG
 901 CCTGGAAGGG GTGATCCAGA CAGCCCCACC TGCAGGAGAG CGTCAGCGCT
 951 GGCAGGGGAG ACAGGCCTTG CCTGCTCCAC CAGCTGCAGG TCCCAGCAG
 1001 GCAGGGAGAG AGGAGAGGTG TGGGGAGCAA GGCAC'TCCCT CCTCTGCCTC
 1051 CCCTCTGAGC AGAGAGATCA GAGTAGGATC ACATGAAAAC GGGGGGGAAA

Figure 5 continues on the following page

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1101 AAAGAGTCTA TTTTGTCTA ATAATAAAGA GTTCTATAA TGTTTAAAAA
1151 AAAAAAAAAA AAA

(vi) DACC-7

1 CGCTCAGGGC ACCTGGTCGG CGAGTTCCCG GCCGGAGGTG TATCTCCATG
51 AATAACTTAA ATGACCCCCC AAATTGGAAT ATCCGGCCCA ATTCCAGGGC
101 TGATGGAGGT GATGGAAGCA GATGGAATTA TGCCCTGTTG GTTCCAATGC
151 TGGGACTAGC TGCTTTTCGC TGGATTTGGT CTCGGGAGTC GAGAAAAGAA
201 ATAGAAAAGG AGAGAGAAGC GTACCGTCAG AGGACGGTTG CCTTCCAGCA
251 GGACCTTGGA GCCAGGTACC ATGCCACAAT TGCAGAAAGC CGGCGGGGCC
301 TGGCACACTT GTCCCTGGAA CTGAAAAGG AGCAGAACAG AACAAC TAGT
351 TACCGAGAAG CCCTCATCTC TCAGGGGCGC AAGTTGGTGG AAGAGAAGAA
401 ACTTCTGGAA CAGGAGCGGG CTCAGGTCCT GCAGGAGAGG AGGCAGCCCT
451 TGCGGAGTGC GTACCTGCGC TGCCTGGGCC AGGAGGAGGA CTGGCAGCGC
501 AGGGCCAGGC TCCTGCTGAG CGAGTTCGAG GCCGCGCTCA CCGAGAGACA
551 GAGCATCTAC TGCAGCCTGG TGCTCCCGCG CCGCAGGCGG CTCGAGCTCG
601 AGAAGAGCTT GCTGGTCCGC GCGTCCACTG ACCCGGTGGC CGCAGACCTC
651 GAGATGGCAG CTGGCCTCAC TGACATATTT AAGCACGATA CGCACTGTGG
701 TGACGTCTGG AACACCAACA AGCGCCAAA CGGGAGGCTC ATGTGGCTGT
751 ATCTCAGATA CTGGGAATA ATCGTTGAAC TGAAGAAGTT TAAGCAGGTA
801 GAGAAAGCCA TACTGGAAAA GTAAGACAGG AGTGAACGGC TCCAGGTCAG
851 AGTCATGGGT TGTGGGTTTT CCGATGTTG CTGCTCCTCC TGCCAGCGCT
901 CCCTAGTTGT GACCGTG CAT GCACACCGCC ACCTCTTAGC AGCGGCCATT
951 CCCGTCACCC TCTGAGGAAG ACAGCAAGGC CTCTGTCCCC TGCAGCAGCT
1001 AAGGACACAG TCTCAGAAGC AGGTCAATAT TTTATTAAGC AGGACAGGAT
1051 AACCTCATAG CTTTAGAGTA AAATTGTTTT TAAGAATATC AAATACAGTG
1101 TTCACCCTAT AAGTCATTCT GTCACCTCCT AAATAAGTTC TGTTTTCTCC
1151 TCAAATTATT TTTCTCTCTC CTAAACTAC AGTTAGAAGT TGTCAGGTAG
1201 CGGTGAGGAC TGCCTCACAG ATGGGAACAG ACGGTGGTGA CGCCAGCAAG

Figure 5 continues on the following page

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1251 GTTTCGTGGT CTGAATCCCA TCAGTGTTTC TTTTTCAC TTGATAACCC
 1301 TTGTGGGTGT TTGGAGTTTG CTGTGCCTGT ATTCAGTAAG CAGATACTGT
 1351 TTATTTAGGT TGGTGCAAAG GTAATTGTGG TTTTGATTG TTGAATTTTG
 1401 CCATTTGATA TTGAAATACA TTCTTAAATA AATGAAGTA TGTTTTGAAA
 1451 GTGCAAAAAA AAAAAAAAAA AAAA

(vii) DACC-8

1 CATTAACGGT GCTAAACATA GACATTTTAA CCCAAGTCAC GACATTCTTA
 51 GCTGTAATC AGCTATCACG GCCTCTTGCT CACCCACTAA TGGTCCCATT
 101 TTCCCCTTGC CGTGTGCACC TCTGCCCATT GTCTTGGTGG CACATGGGTG
 151 GAACACTTGA TCTGCTCGAG TCTGCCTTCA ACACACGTTG CATCTTCAGA
 201 TTTTCTACTT TTCTGCTTGA AACTAATATT CACCAAGTCA GACTTTGTGT
 251 TAACCTTTATT TCAGGGTATT GGCTGCCAGG GGGTCATTCC TAAGTGGCCT
 301 GAAGATGGAC AAAGGGAAGT AACAGGCACG TGATGTTGGC AAGGATGCTT
 351 CTAGGGCTAG AGGATCAGTG GTGGGAGAGA CCTGCAGAAT CTACCAGCCA
 401 GAACCGCAGA TAACAAATCT TGTGGTCAGG GGCTGTGACT GAGAGAAGGA
 451 AATTGAGGCT GTGTTCTGGA AGTACATATA AACTTCTCAC ACAAAACCGA
 501 TTCTTCACCA TTTCCCTTTC TCACTTTGCA GTGCCATTTC TTTTGCATT
 551 AGGCAATTTG CTCAGACTTT TCAGAGCCAC GGCCCATCCG TTCTCTGGAA
 601 TCCCCCACAC CTCTGAGAGG TGGATCACCA CATCCTGCAG GGCTGCTCCC
 651 CTCCAAACTA CCTTTCGGAG ATGCAGGACA GGGAGGCTGT TTCAGCCAGA
 701 AAGACCAAAA TCAAGAGCGA GGTGCAGAAC GTGGTAAAC AGAAAAAGGG
 751 CAGGTGGCAA ATTGGTTTTT TTTTGGGTTT TCTGGTTTTT TTTTTTCCA
 801 CATCTGGATG GCTGTCACCA GAGATCTTTC CTTCAGTCGC TAGCATGTTT
 851 CTCCTCTTCT CCCCTCCCCA CTTTTTCTTT CTATTAATCA AAAGAAATTT
 901 CAAAATCAAT GGGATGGTCG GATCTCACAG GCTGAGAACT CGTTCACCTC
 951 CAAGCATTTT ATGAAAAAGC TGCTTCTTAT TAATCATGCA AACTCTTGCC
 1001 ACGATGTGAA GAGTTTGACA AATCTTTCAA AATAAAAAGT ACTGATTAG
 1051 AAACTGAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA

Figure 5 continues on the following page

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(viii) DACC-9 (5' end)

1 CGACGGCGGA GCAGGATGGA GATCCCGGTG CCTGTTGAGC CGTCTTGGCT
 51 GCGCCGCGCC TCGGCCCTT TGCCTGGGCT GTCGGCTCCC GGGCGCCTCT
 101 TCGACCAGCG CTTGCGCGAG GGGCTGCTGG AGGCCGAGCT GGCTGCGCTC
 151 TGCCCTGCCG CGCTGGCCCC CTACTACCTG CGCGCACCCA GCGTGGCGCT
 201 GCCTACCGCC CAGGTATCGA CCGACCCCGG GCATTCTCTG GTGCTGCTGG
 251 ATGTGAAACA CTTCTCACCC GAGGAAATTG CCGTCAAGGT GGTGGTGAC
 301 CACGTGGAGG TTCATGCGCG CCACGAGGAG CGCCCGGATG AGCACGGATA
 351 CATTGCGCGC GAGTTCACGC GGCTACCGCT TGCCGCTGGC GTGGACCCTG
 401 CGGCCGTGAC

(viii) DACC-9 (3' end)

1 TCATCCCCTC ACCCATTTT AATCCCACCC ACCACCAAAG ATTATGGTGT
 51 AGGCAAGCCC TGCCCCCACC CTAGGCCAGT CAAGCATAAT CCCCCCTTCT
 101 CAGATGTCCA AGACCCGTGC ACAGACCTCC TACCCCGGAC CATCCTGGCC
 151 TGGTCCNCAA GACTGGATCC TTCCCCTCAT TCCAACCAGA TACACTTCTC
 201 CTCACCTCTT CCCTTCAACC CATTCTCTAA CCTGAAACCT CAGCCAGCCA
 251 CTCCCAGATC CTTGAACCCC TTTTCTGACC CTACCCGTGT ACCCCTATTC
 301 TAAGCCAACC AGAACCCTCA ACCTCAAACCT GTATAGATAC CCATCCCTCC
 351 TCCCCAGAGT CTGCACAGAT ATCCACGCT ATCCAGAACT CCTCAGTCAC
 401 TCTGTCTTGA CCCCCCAAAT CTCCAACCAC ACCACCCCTC CCCTTATTCT
 451 CCAAGACCCA ACCAAGCAGC CACTTTCTTT AATTCCCTAC AATCTTCTC
 501 CCTCCTCAAA TTCCCTGATG CCCCATCCCC CCACCTAGGC CCACTCCCCC
 551 AATAAATGTG CTAGAGCTAA AAAAAAAAAA AAAAAAAAAA

(ix) DACC-10

1 AGGGGATCGA GGTGAGAAGG GTCACAGAGG CTTTACTGGT CTTCAAGGTC
 51 TTCTTGGACC TCCTGGTCCA AATGGTGAAC AAGGCAGTGC TGAATCCCT
 101 GGACCATTTG GCCCAAGAGG CCCCCAGGT CCAGTTGGTC CTTAGGCAA
 151 AGAAGGAAGC CCTGGGCCGC TTGGGCCCAT TGGGCCTCCT GGTGTGCGGG

Figure 5 continues on the following page

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201 GCAGCGTTGG AGAAGCAGGC CCTGAGGGTC CTCCTGGTGA GCCTGGTCCC
251 CCCGGCCCTC CGGGACCCCC TGGCCACCTT ACAGCTGCTC TTGGGGATAT
301 CATGGGGCAC TATGATGAGA GCATGCCAGA CCCACTTCCG GAGTTTACTG
351 AAGATCAGGC GGCTCCTGAT GACAAAAACA AAACCGACCC CGGGGTACAT
401 GCGACCCTGA AGTCACTCAG TAGTCAGATT GAAACCATGC GTAGCCCTGA
451 TGGCTCTAGA AAGCACCTG CCCGGACCTG TGACGACTTA AAGCTTTGCC
501 ATTCTGCAAA GCAGAGCGGT GAGTACTGGA TTGACCCTAA CCAGGGATCT
551 GCTGAAGATG CAATCAAAGT TTA CTGCAAC ATGGAAACAG GAGAAACGTG
601 TATTCAGCA AATCCATCCA GTGTCCACG GAAAACCTGG TGGGCCAGCA
651 AATCTCCTGA TAATAAGCCT GTTTGGTATG GTCTTGATAT GAATCGAGGA
701 TCTCAGTTTG TTTATGGAGA CCACCAGTCA CCTAATGCAG CCATTACTCA
751 GATGACCTTC TTGCGCCTTT TATCGAAAGA AGCCTCCAG AACATCACCT
801 ACATCTGTAA AAACAGTGTA GGATACATGG ATGATCAAAC TAAGAACTTG
851 AAGAAAGCTG TGGTTCTCAA AGGGTCAAAT GACTTAGAAA TCAAAGCAGA
901 GGGAAATGTT AGATTCAGAT ACATAGTTCT TCATGATTCT TGCTCTAAAC
951 GAAATGGAAA CGTGGGCAAG ACCATCTTTG AATATAGAAC ACAGAATGTG
1001 GCACGCTTGC CCATCATAGA TCTTGCCCCT GTGGATGTTG GCAGTACAGA
1051 CCAAGAATTT GGCATAGAAA TTGGACCAGT TTGTTTTGTG TAAAGCAAGC
1101 CGAGATACAT CGACAATGAG CACCACCCCT ACCATCAGTG ACCACCACCA
1151 TTCACAAGAC TTTGACTGTT TGAAGCTGAT CCTGAGACTC TTGAAGTAAT
1201 GGCTGATTCT GCATCAGCAT TGTATATATG GTCTTAAGTG CCTGGCCTCC
1251 TTATCCTTCA GAATATTTAT TTTACTTACA GTCCTCAAGT TTTAATTGAT
1301 TTAAAATATT TTTCAATACA ACAGTTTAGG TTAAAATGA TCAATGACAA
1351 AGACCACCTT TTA AAAAAA AGTAACTGA TTGAATAAAT AAATCTCCGT
1401 TTTCTTCATT TCAGTGTAAT GACAAAGTTG CTTAGTATTT ATGAGAAAAA
1451 CTTTCTTCCT GGCAGATAGC TTAAAGAGTG GGGTATATAA AATCACAACA
1501 CTTTTATTTT ACGTGGCTGC AATTGGAAAA ATACAAAGTA ATGCCCTTTT
1551 GTGACCTCTC ATTTACAGAT TATCAATTAA AAATGAAATC AAAATGTGAA

Figure 5 continues on the following page

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1601 AAAAAAAAAA AAAAAAAAAA AAAAA

(x) DACC-11

1 CCTGGTGCTC CTGGCGCTCC CGGTGCCCCC GGCCCTGTCTG GACCTGCTGG
51 CAAGAGCGGT GATCGTGGTG AGACTGGTCC TGCTGGTCCT GCTGGTCCCA
101 TTGGCCCCGT TGGTGCCCGT GGCCCCGCTG GACCCCAAGG CCCCCGTGGT
151 GACAAGGGTG AAACAGGCGA ACAGGGCGAC AGAGGCATTA AGGGTCACCG
201 TGGCTTCTCT GGTCTCCAGG GTCCCCCTGG CCCTCCCGGC TCTCCTGGTG
251 AGCAAGGTCC TTCCGGAGCC TCTGGTCCTG CTGGTCCCCG CGGTCCCCCT
301 GGCTCTGCTG GTACTCCTGG CAAAGATGGA CTCAATGGTC TCCCAGGCC
351 CATCGGTCCC CCTGGGCCTC GAGGTCGCAC TGGTGATGCT GGTCTGCTG
401 GTCCTCCCGG CCCTCCTGGA CCCCCTGGTC CCCCCTGGTCC TCCCAGCGGC
451 GGCTACGACT TAAGCTTCCT GCCCCAGCCA CCTCAAGAGA AGGCTCACGA
501 TGGTGGCCGC TACTACCGGG CTGATGATGC CAATGTGGTC CGTGACCGTG
551 ACCTCGAGGT GGACACCACC CTCAAGAGCC TGAGCCAGCA GATCGAGAAC
601 ATCCGGAGCC CTGAAGGCAG CCGCAAGAAC CCCGCCCCGA CCTGCCGTGA
651 CCTCAAGATG TGCCACTCTG ACTGGAAGAG CGGAGAATAC TGGATTGACC
701 CCAACCAAGG CTGCAACCTG GATGCCATTA AGGTCTTCTG CAACATGGAA
751 ACTGGTGAGA CCTGTGTGTA CCCCCTCAG CCCATCGTGG CCCAGAAGAA
801 CTGGTACATC AGCAAGAACC CCAAGGACAA GAGGCACGTC TGGTACGGCG
851 AGAGCATGAC CGGCGGATTC CAGTTCGAGT ACGGCGGCCA GGGCTCCGAT
901 CCTGCCGATG TGGCCATCCA GCTGACTTTC CTGCGCCTGA TGTCCACCGA
951 GGCCTCCCAG AACATCACCT ACCACTGCAA GAACAGCGTG GCCTACATGG
1001 ACCAGCAGAC TGGCAACCTC AAGAAGGCC TGCTCCTCCA GGGCTCCAAC
1051 GAGATCGAGA TCCGGGCGCA GGGCAACAGC CGCTTCACCT ACAGCGTCAC
1101 CTACGACGGC TGCACGAGTC ACACGGAGC CTGGGGCAAG ACAGTGATCG
1151 AATACAAAC CACCAAGACC TCCCGCTTGC CCATCATCGA TGTGGCCCCC
1201 TTGGACGTTG GCGCCCCAGA CCAGGAATTC GGCTTCGACG TTGGCCCTGT
1251 CTGCTTCCTG TAAAGTCCTT CCACCCCAAC CTGGCTCCCT CCCACCCAAC

Figure 5 continues on the following page

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1301 CCACTTGCCC CTGACTCTGG AACAGACAA ACAACCCAAA CCGAAACCCC
1351 CAAAAAGCCA AAAAATGGGA GACAATTTCAT CATGGACTTT GGAAAATATT
1401 TTTTTCCTTT GCATTCATCT CTCAAACTTA GTTTTATCT TTGACCAACT
1451 GGACATGACC AAAAACCAAA AGTGCATTCA ACCTTACCAA AAAAAAAAAA
1501 AAAAAAAA

Figure 5

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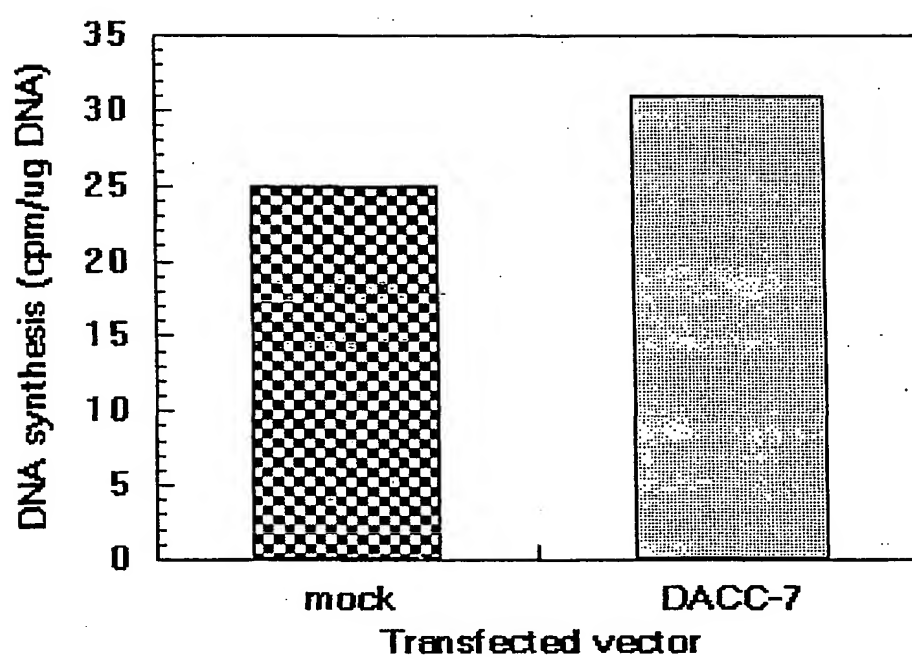


Figure 6

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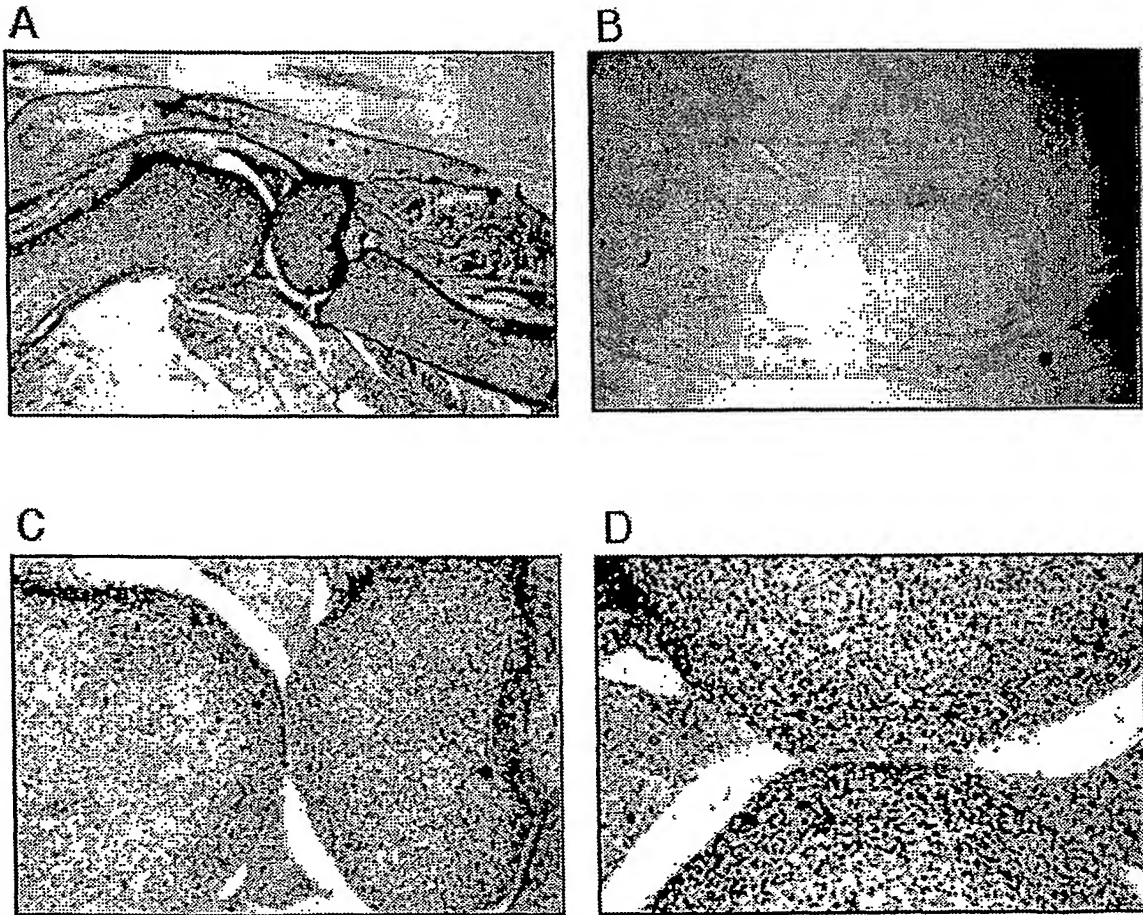


Figure 7

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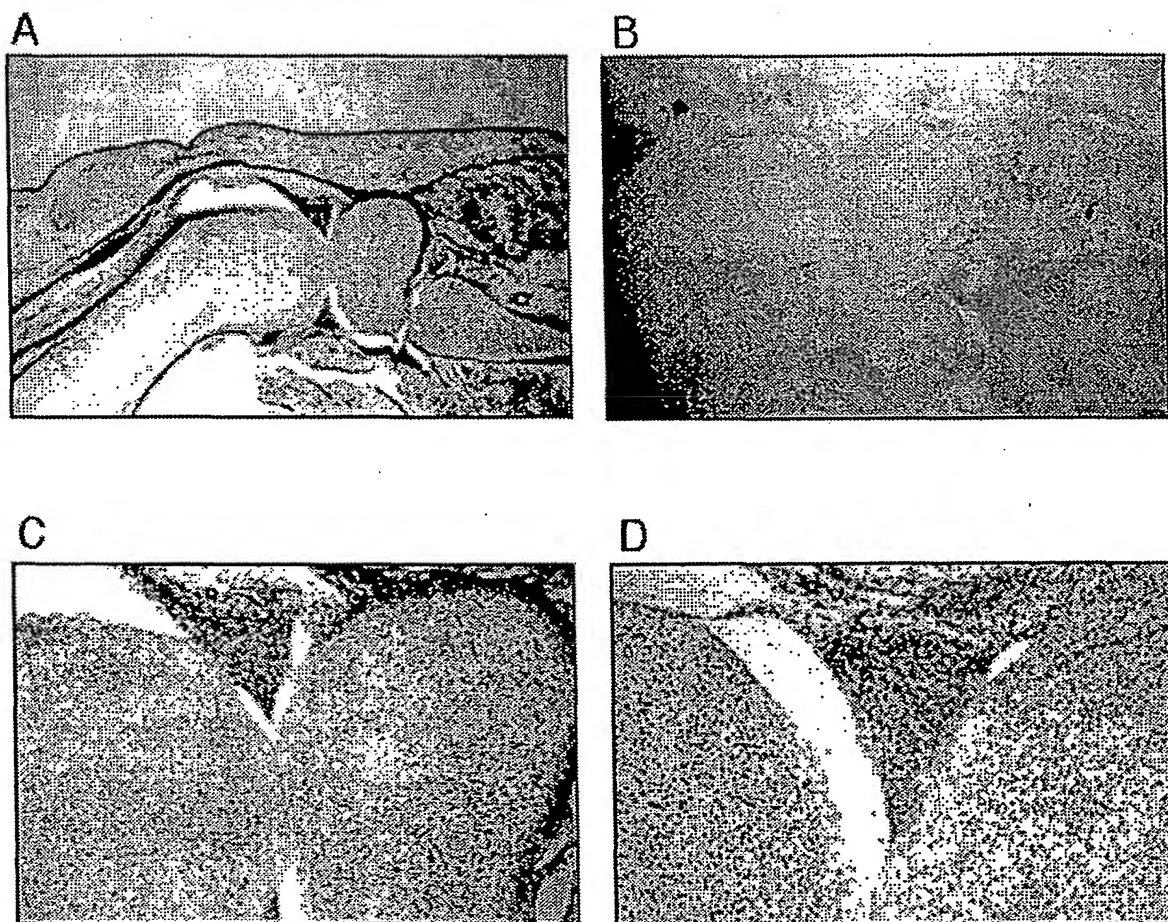


Figure 8

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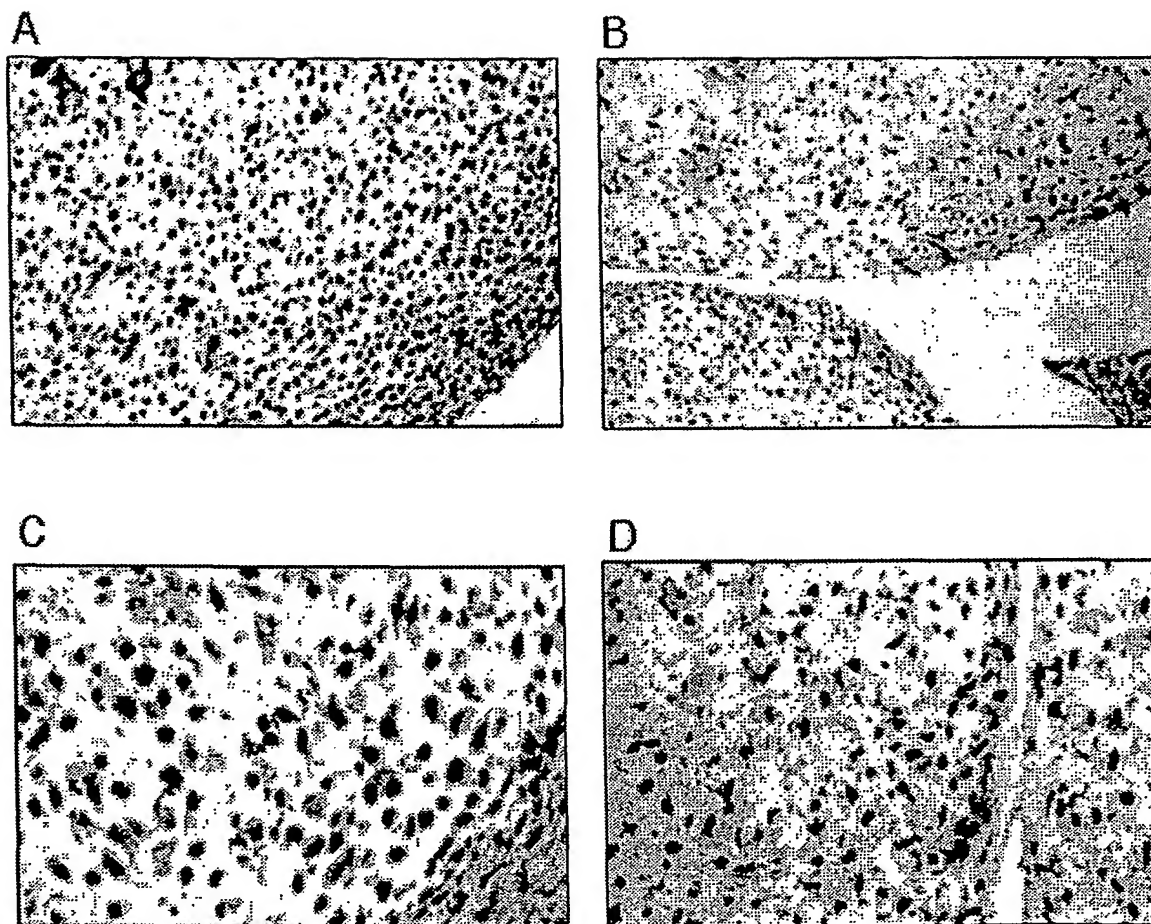


Figure 9

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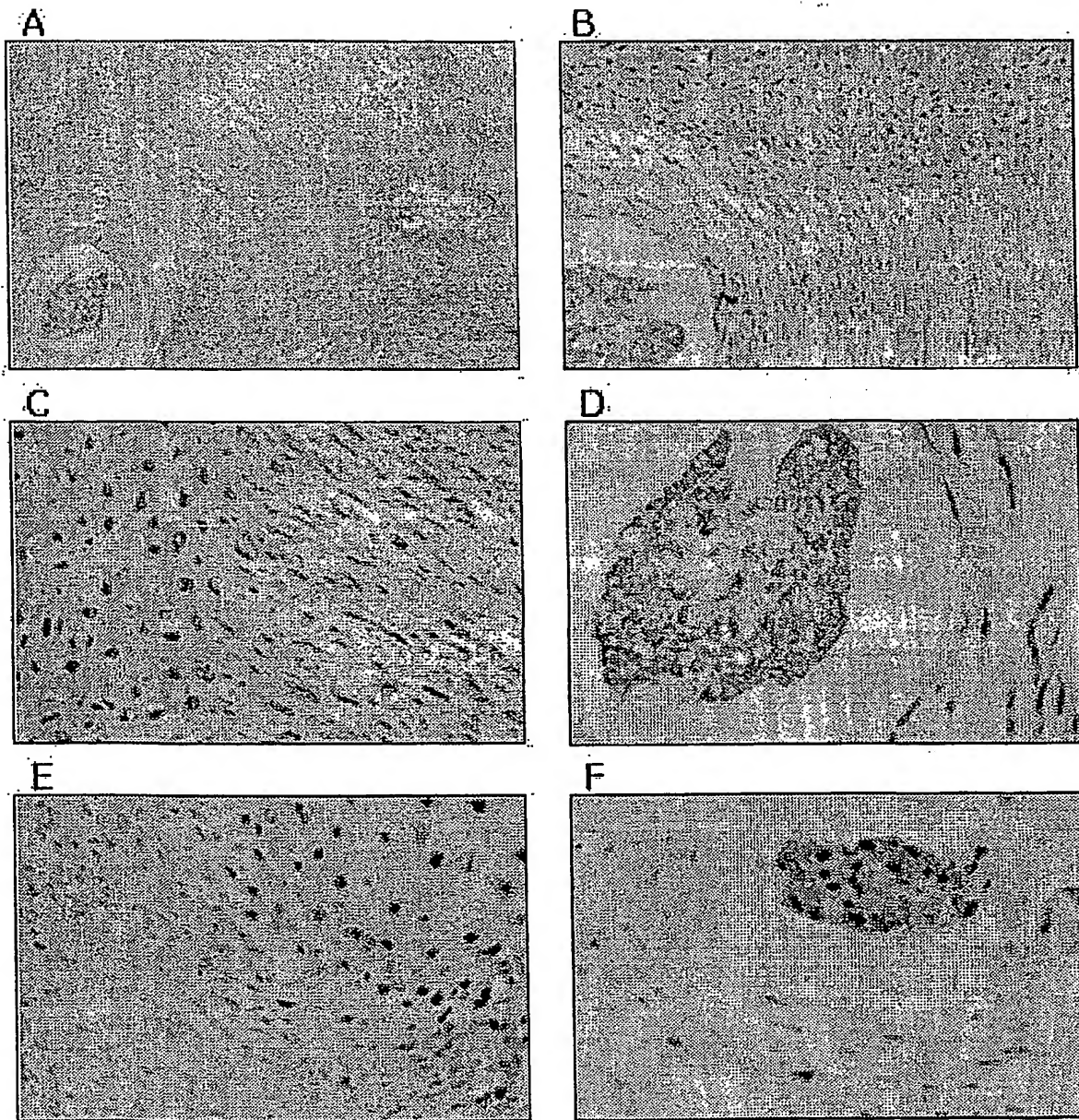


Figure 10

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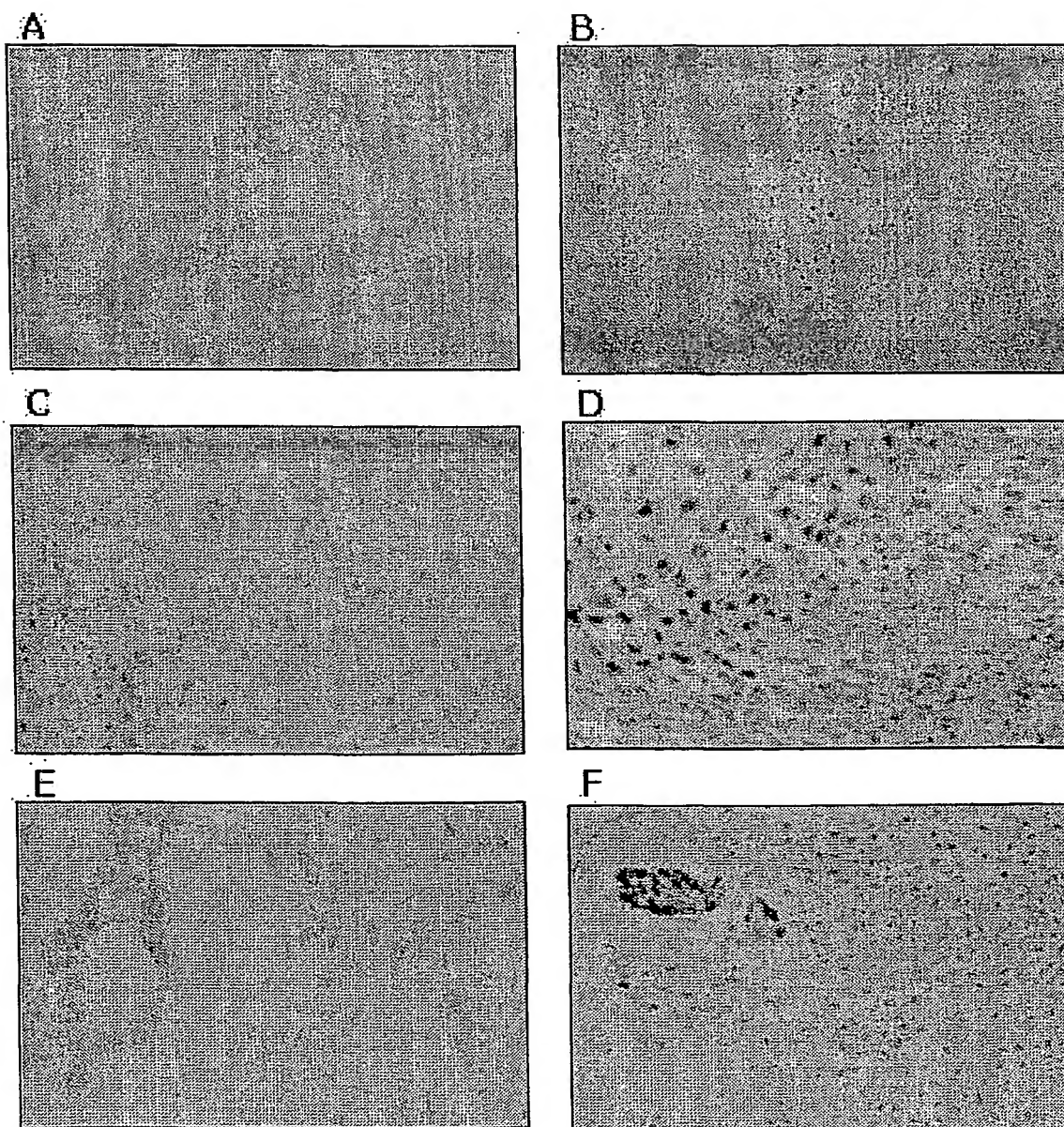


Figure 11

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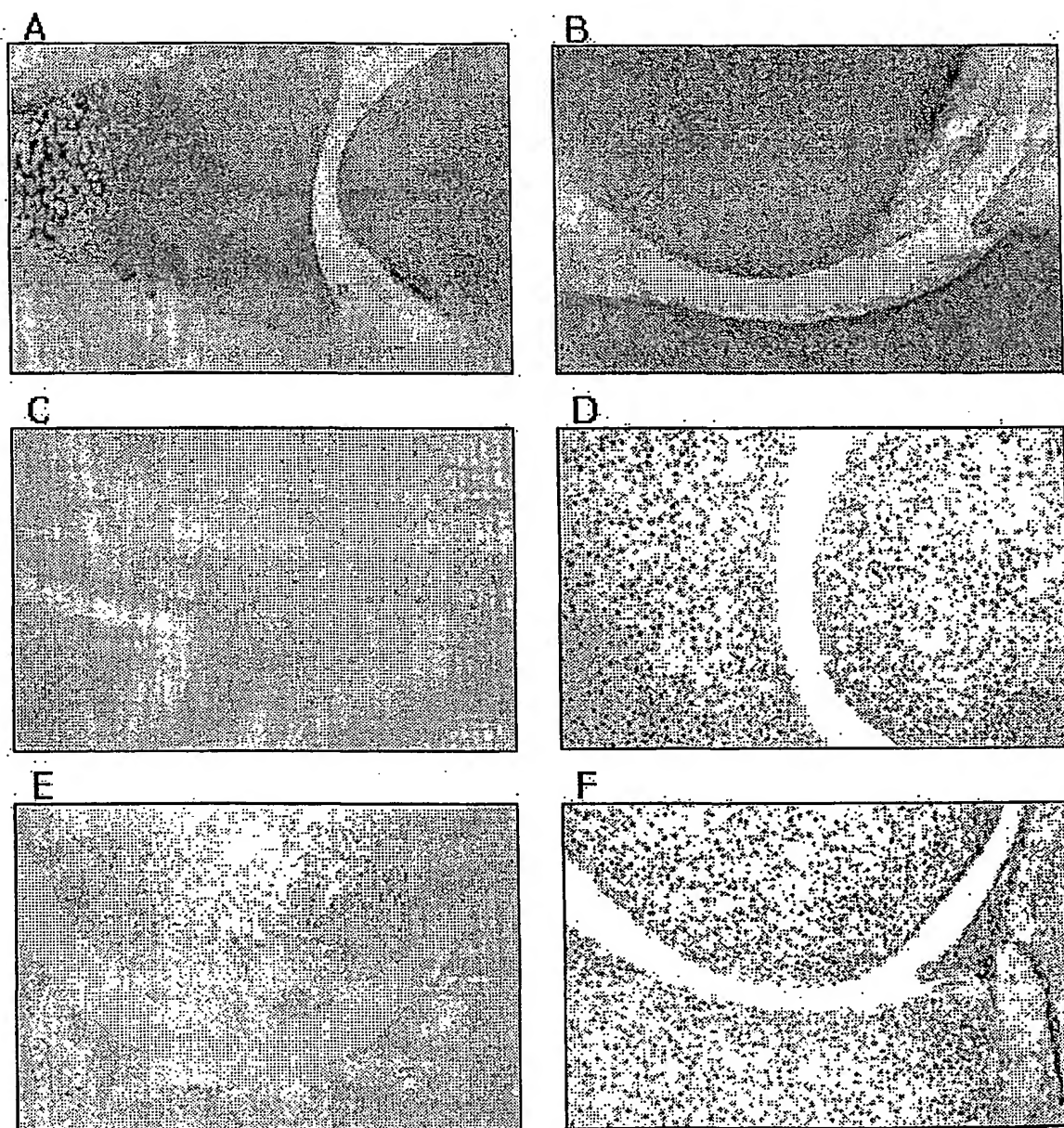


Figure 12

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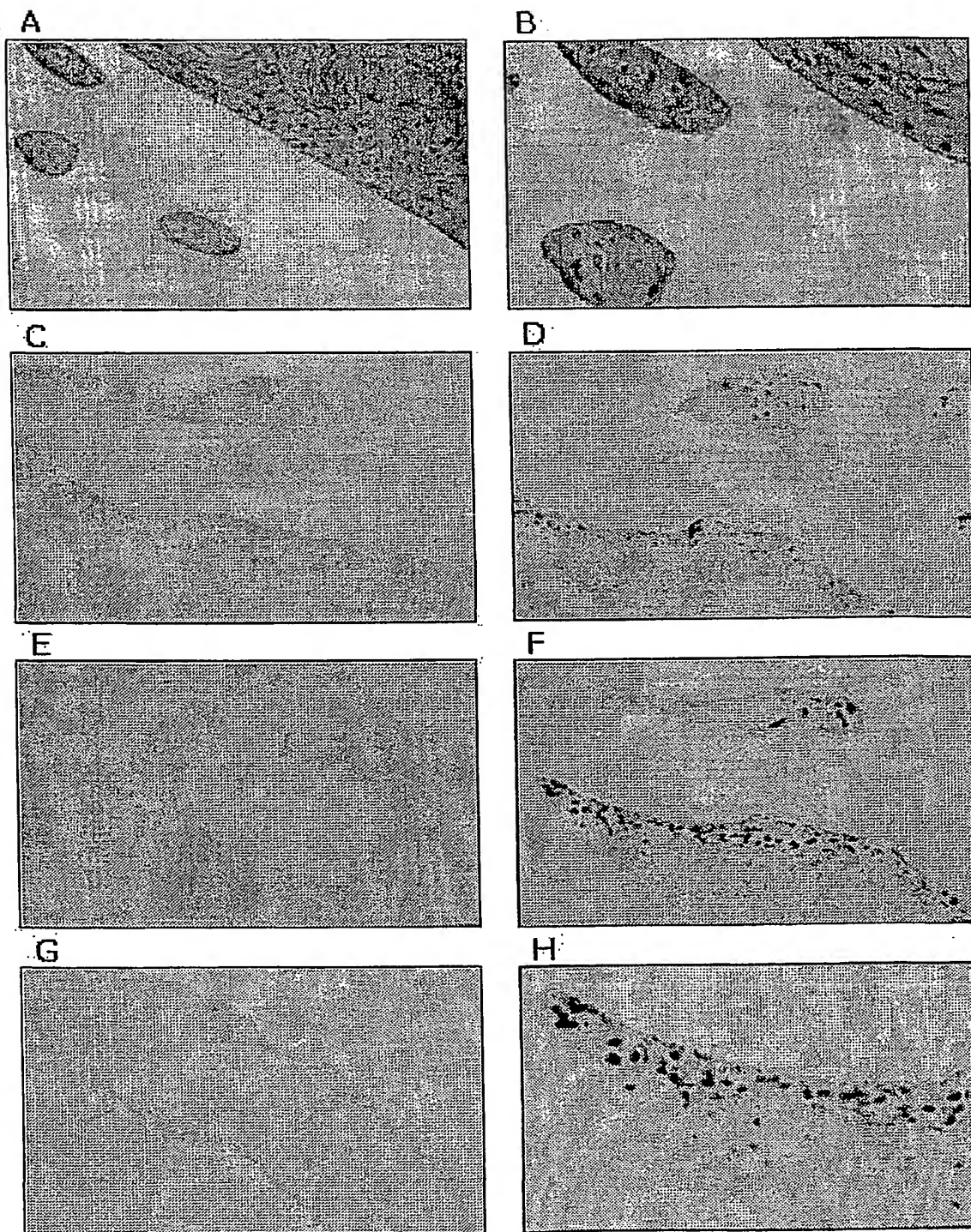


Figure 13

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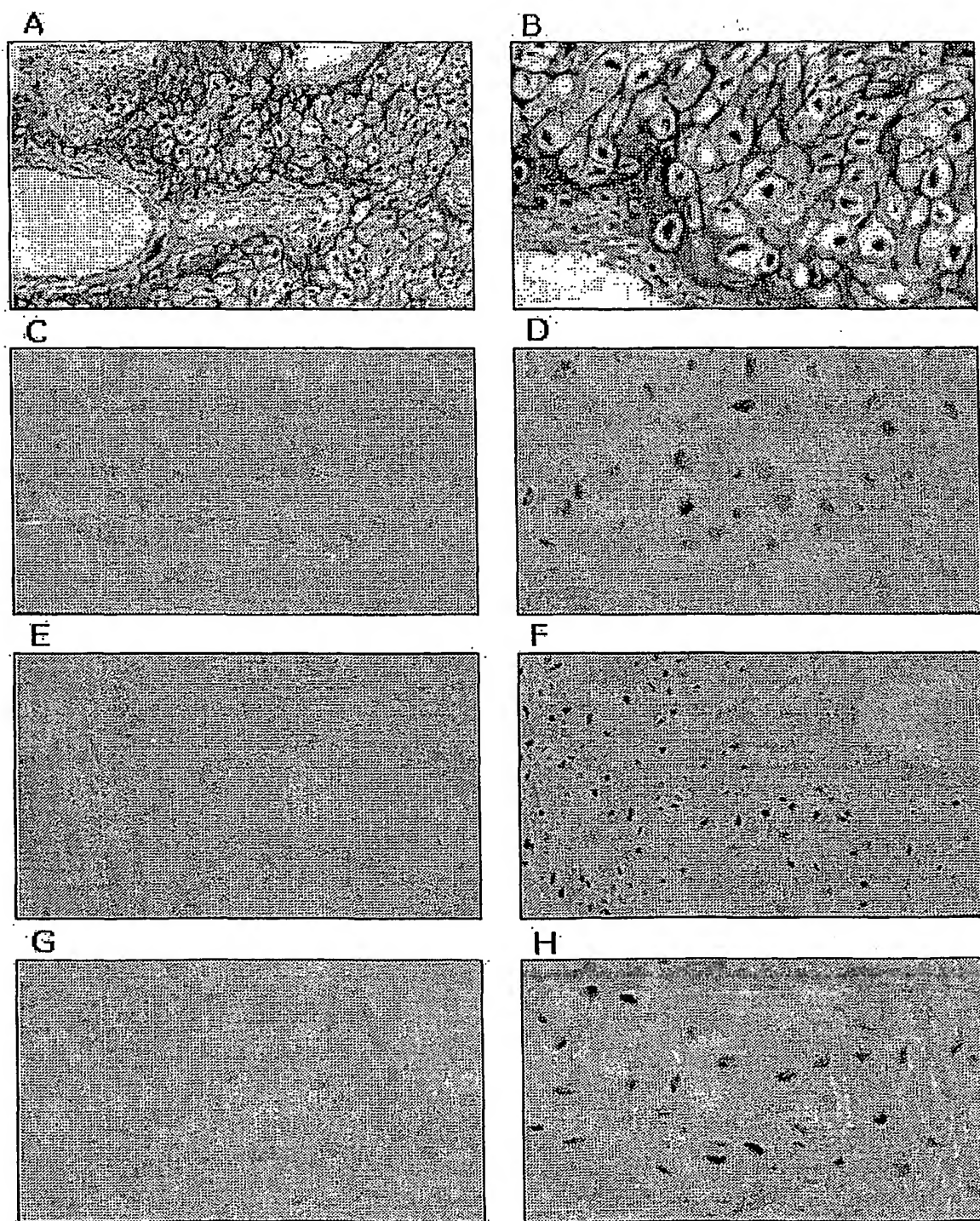


Figure 14

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Deduced amino acid sequence of DACC-7

```

1  MNNLNPPNWNIRPNSRADGGDSRWNYALLVPMGLAAFRWIWSRESRKEIEKEREAYR
61  QRTVAFQQDLGARYHATIAESRRVAHLSLELEKEQNRTTSYREALISQGRKLVEEKLL
121 EQERAQVLQERRQPLRSAYLRCLGQEEDWQRRARLLLSEFEAALTERQSIYCSLVLPRRR
181 RLELEKSLLVRASTDPVAADLEMAAGLTDFKHDTHTCGDVWNTNKRQNGRLMWLYLRYWE
241 LIVELKKFKQVEKAILEK*

```

The asterisk indicates the stop codon.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
Residue																				
Count	23	3	10	27	5	10	4	9	14	35	4	11	7	14	32	14	9	11	8	8
%	8.88	1.15	3.86	10.42	1.93	3.86	1.54	3.47	5.4	13.51	1.54	4.24	2.7	5.4	12.35	5.4	3.47	4.24	3.08	3.08

Length: 258 amino acids

Mass: 30.503 kDa

pI: 10.29

	Human LOC133957	Mouse RIKEN 0610011N22
Amino acid identity	90%	83%
Amino acid similarity	97%	92%

Figure 15

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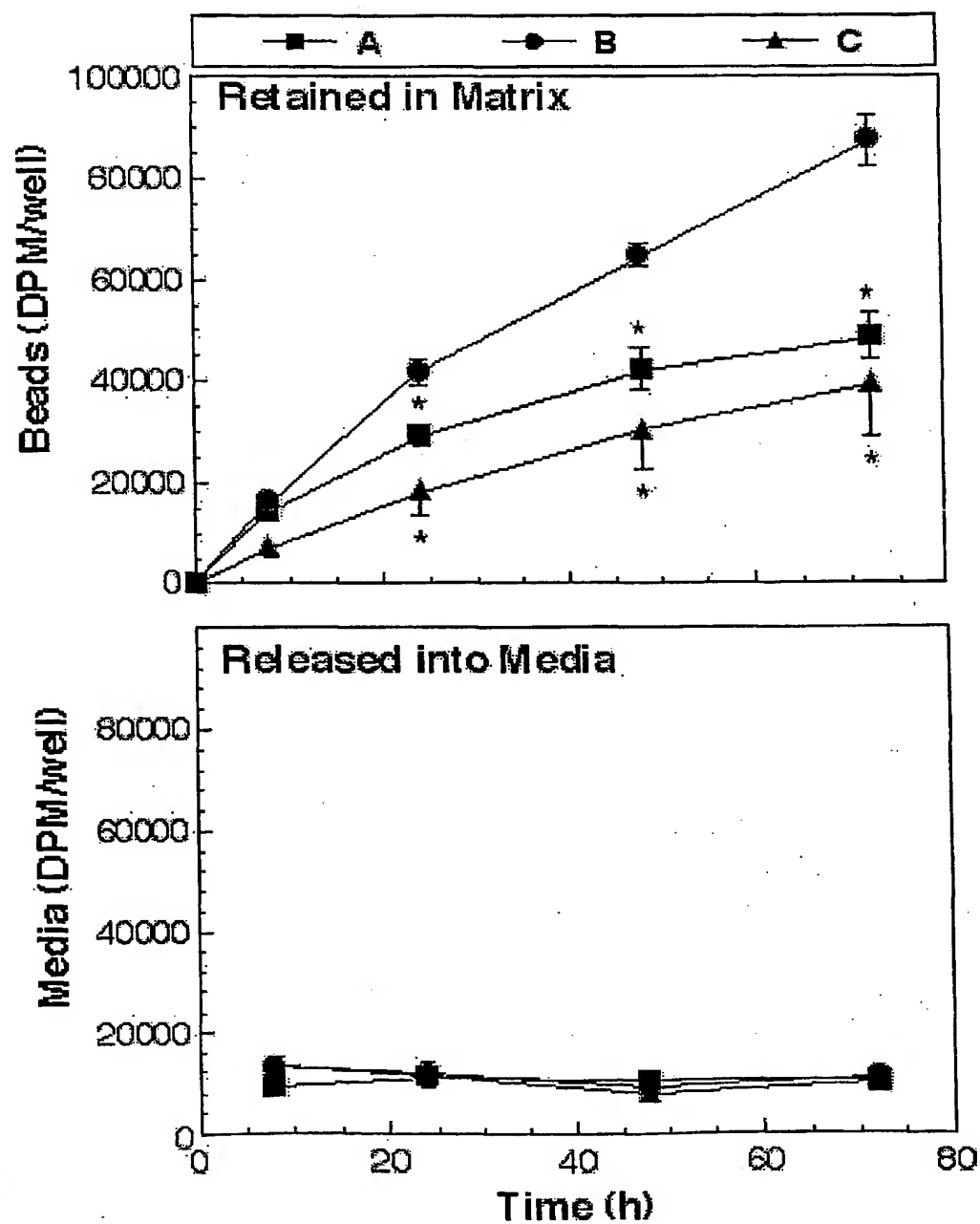


Figure 16

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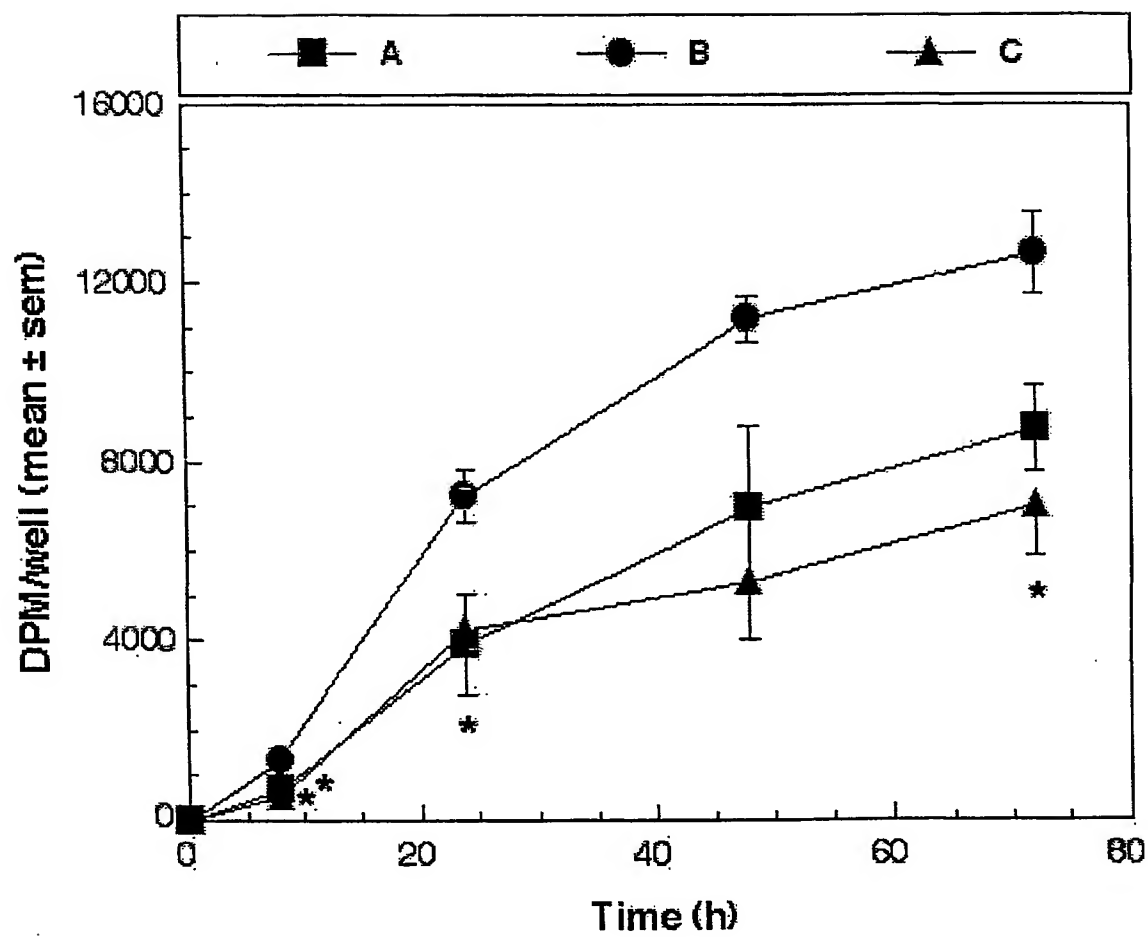


Figure 17

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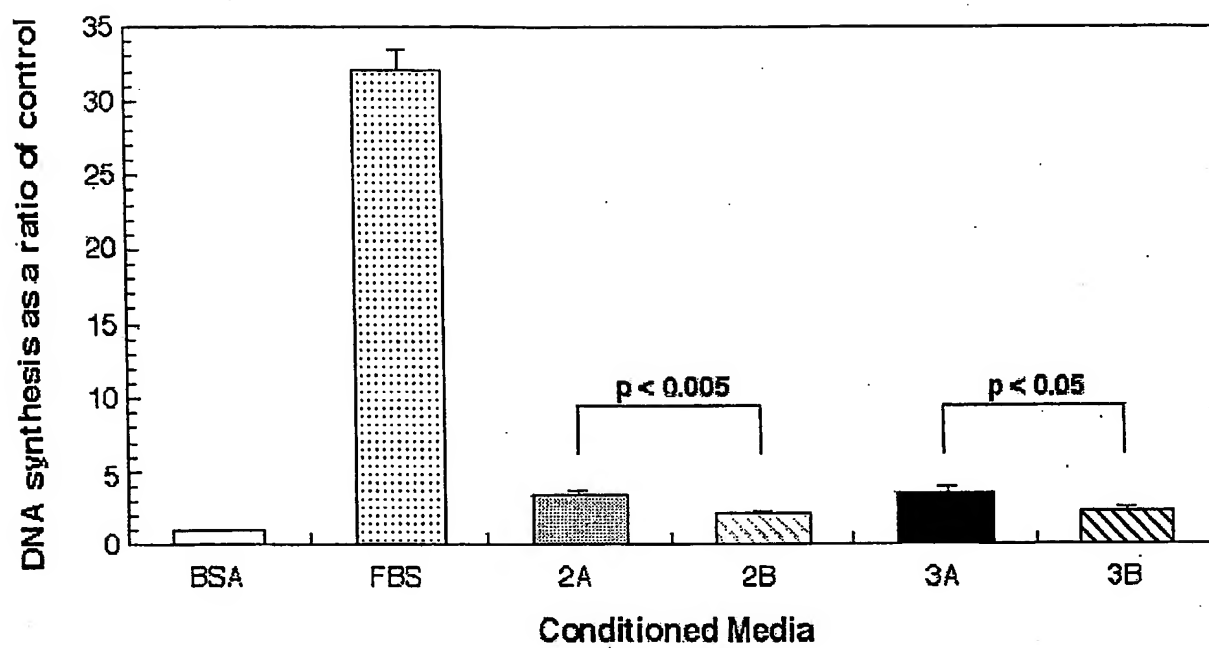


Figure 18

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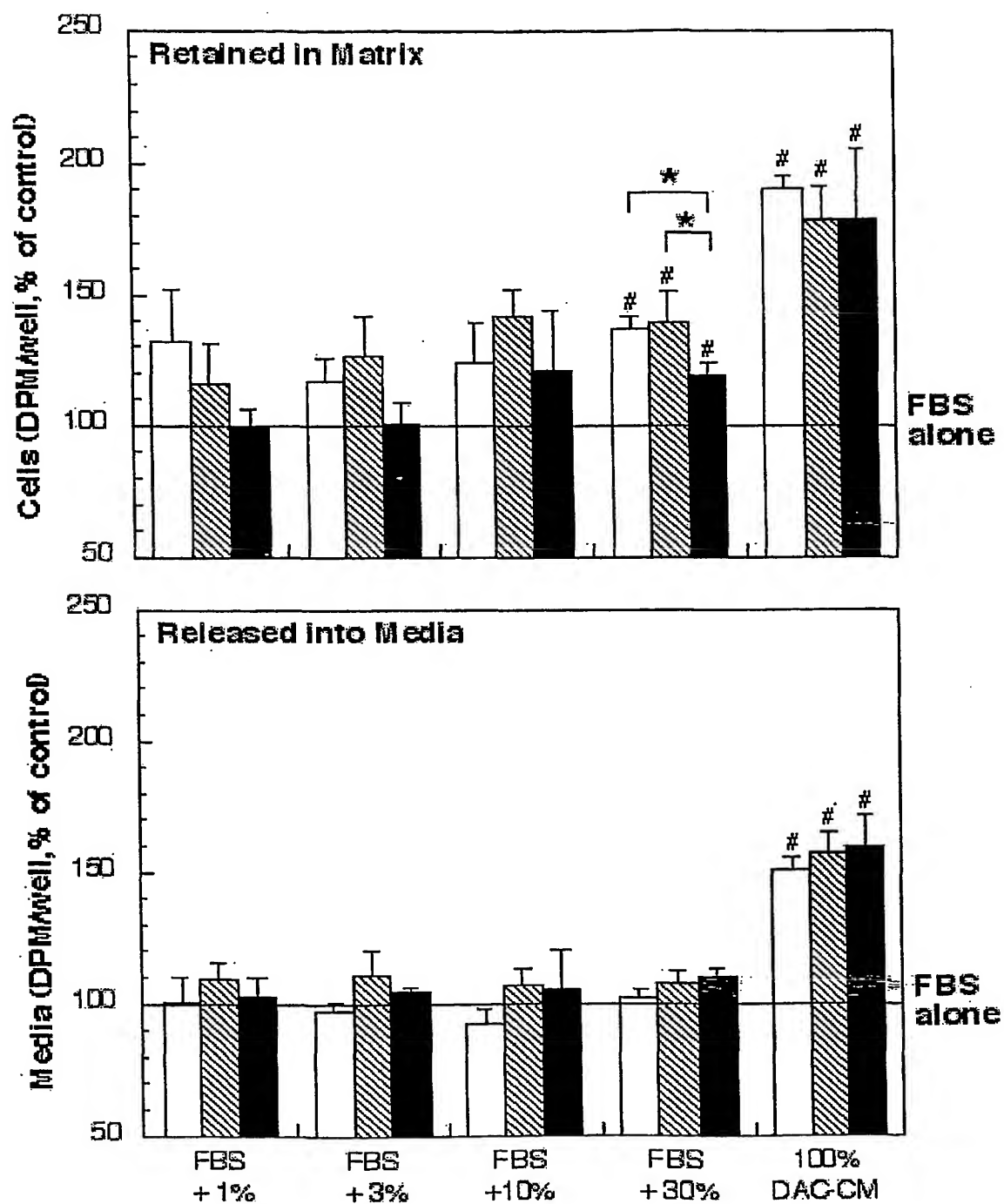


Figure 19

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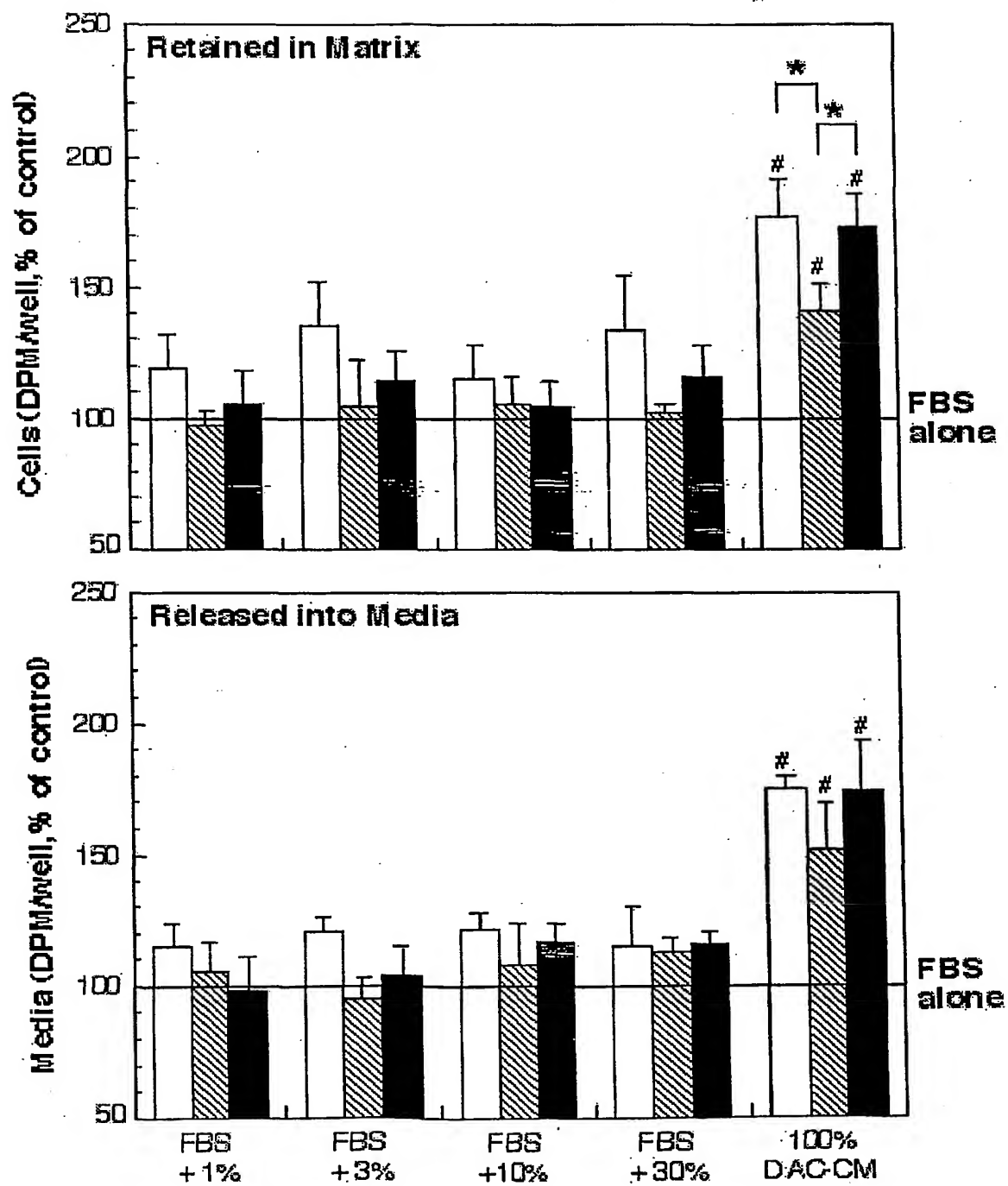


Figure 20

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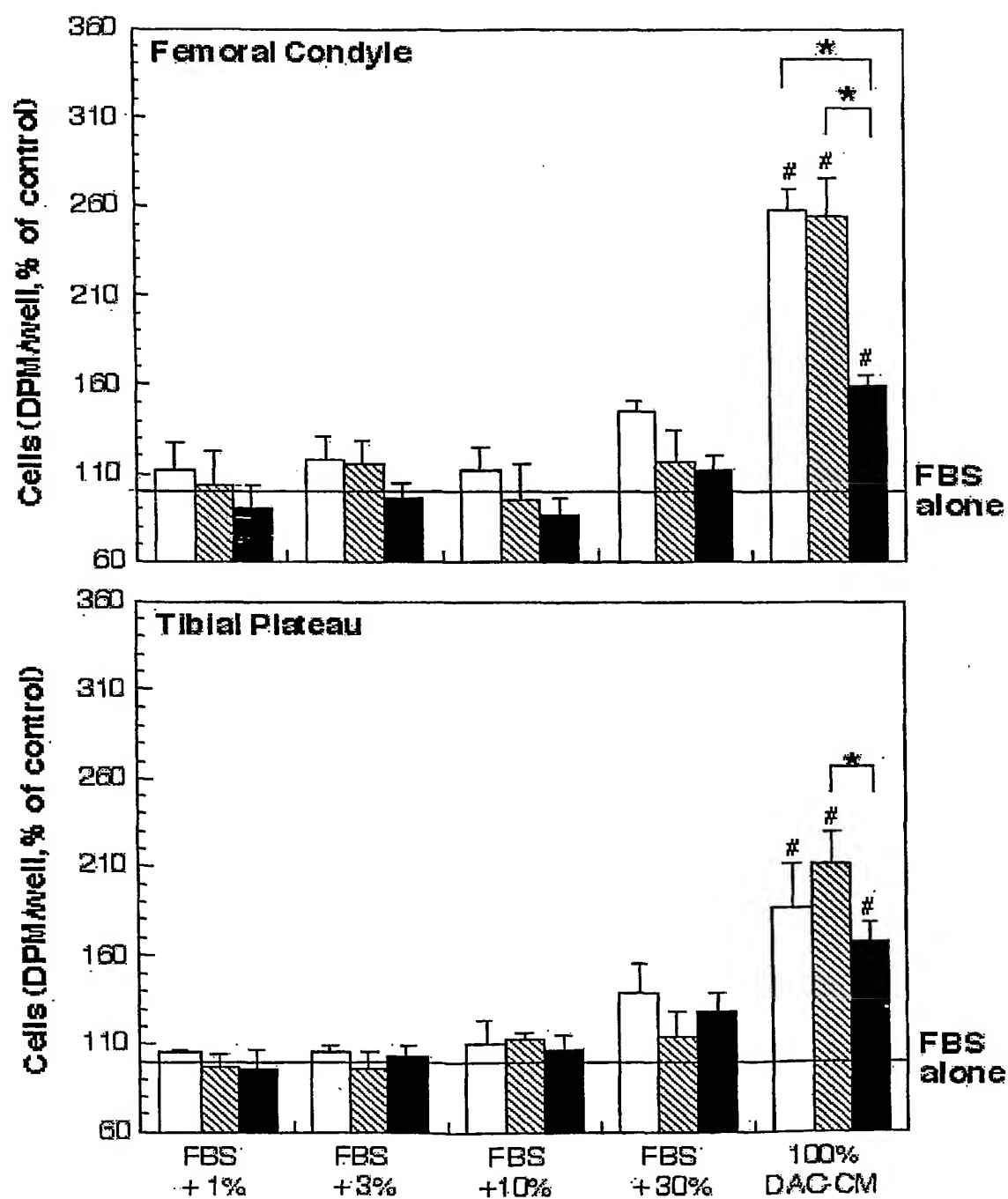


Figure 21

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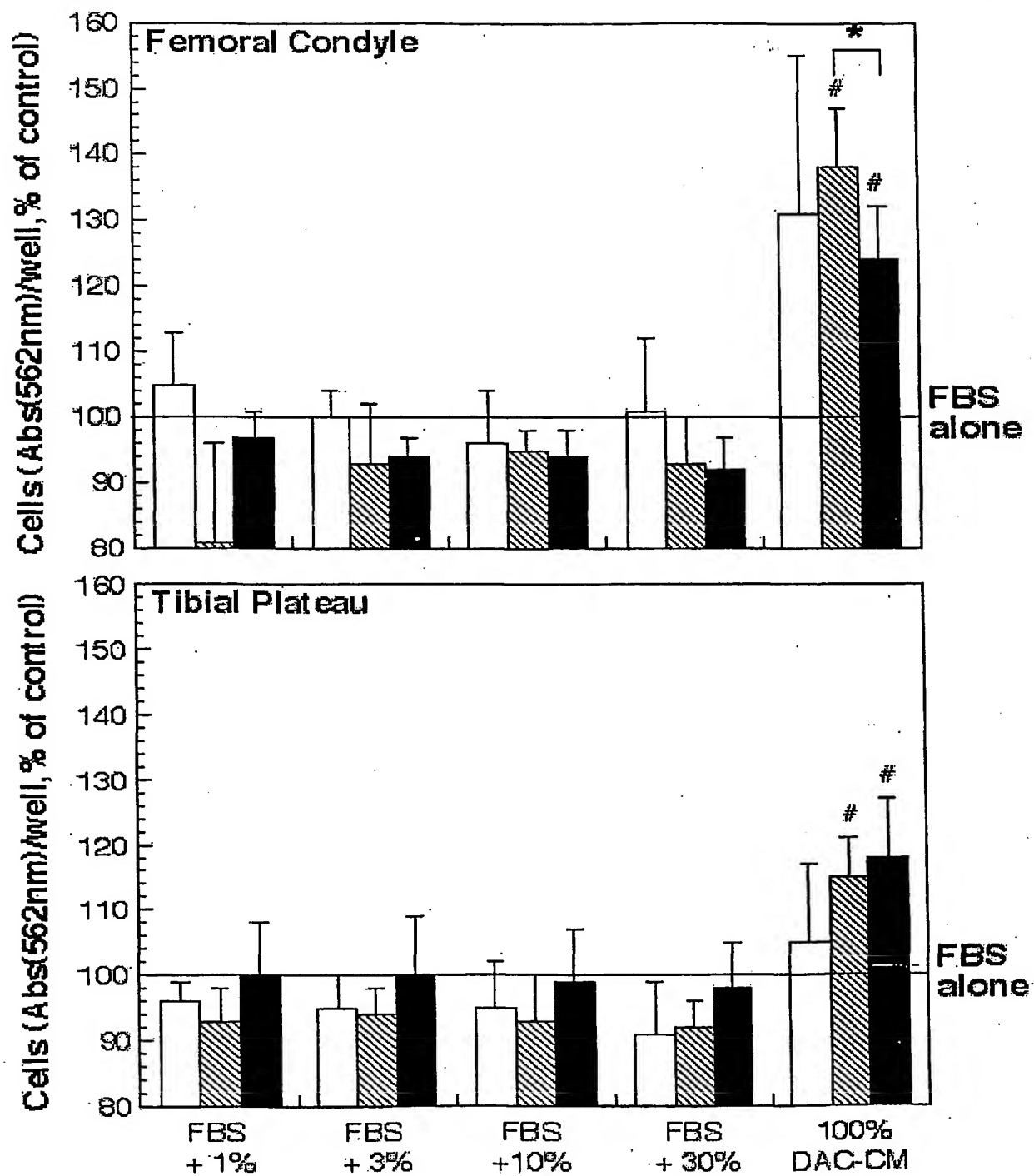


Figure 22

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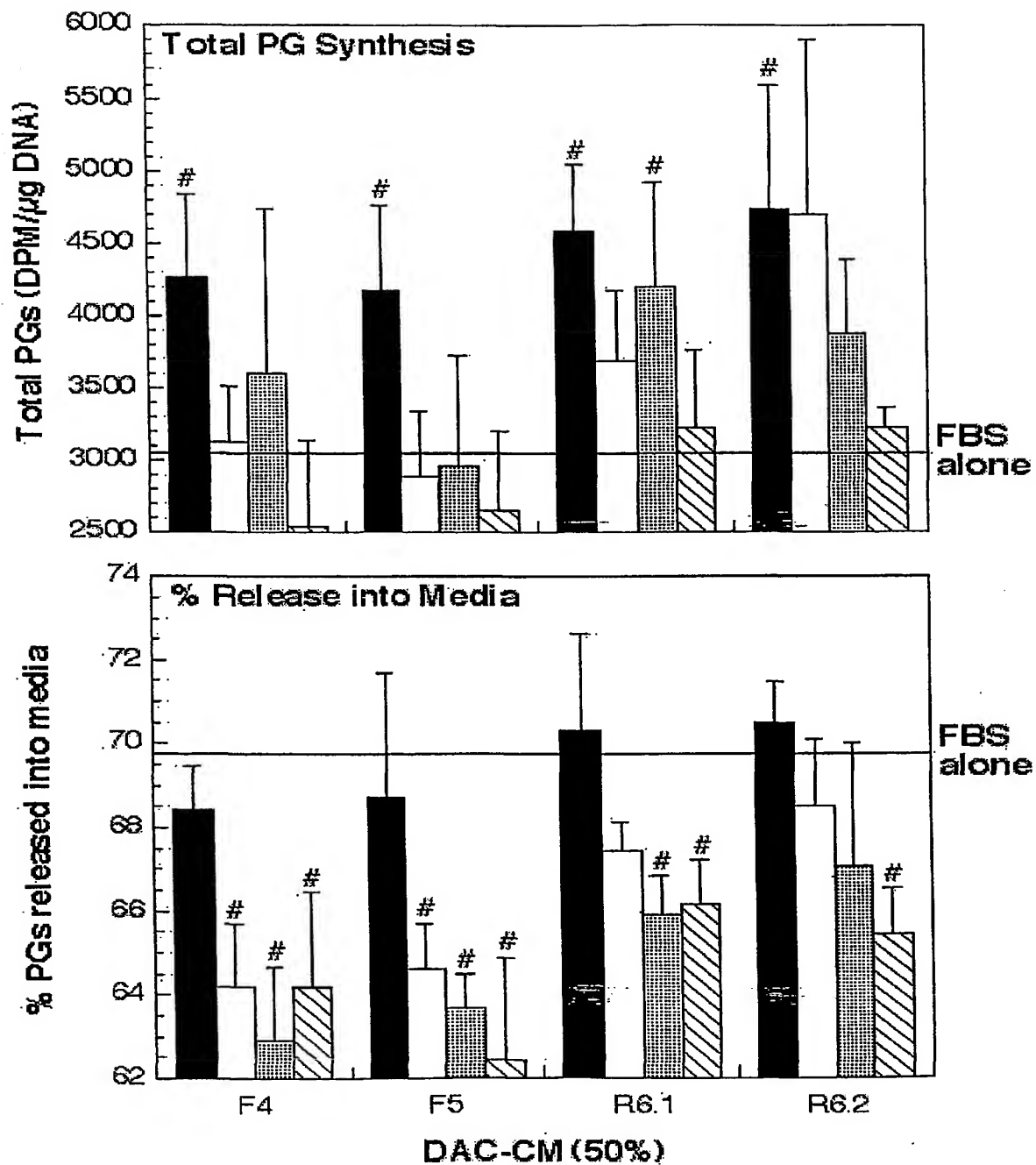


Figure 23

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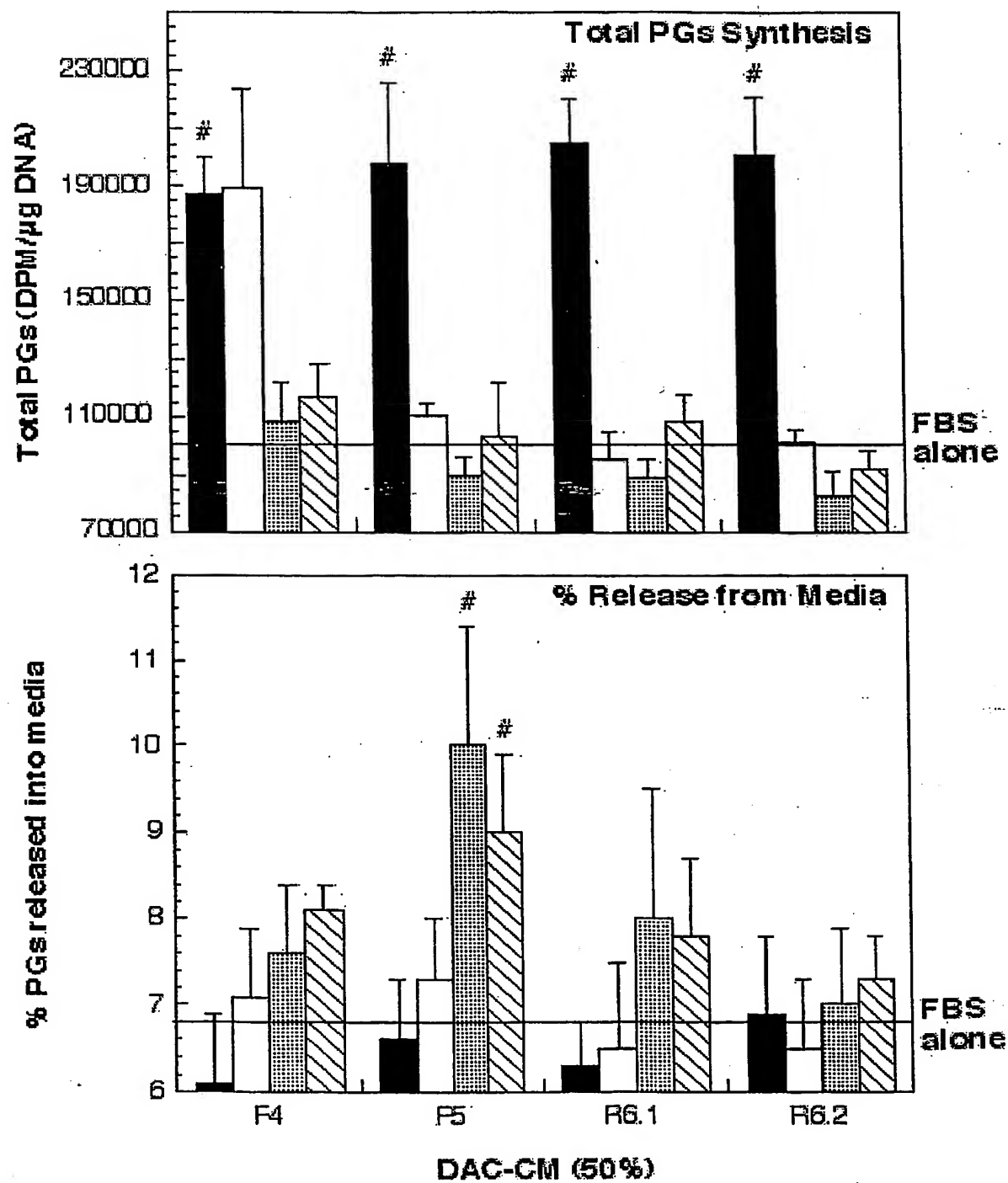


Figure 24

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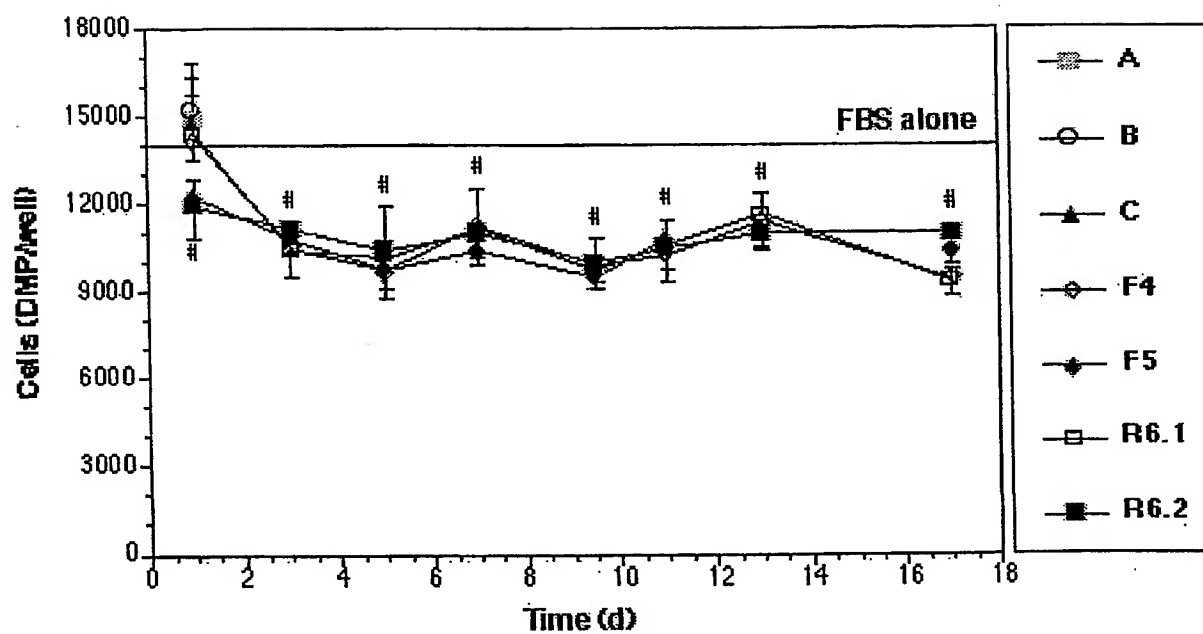


Figure 25

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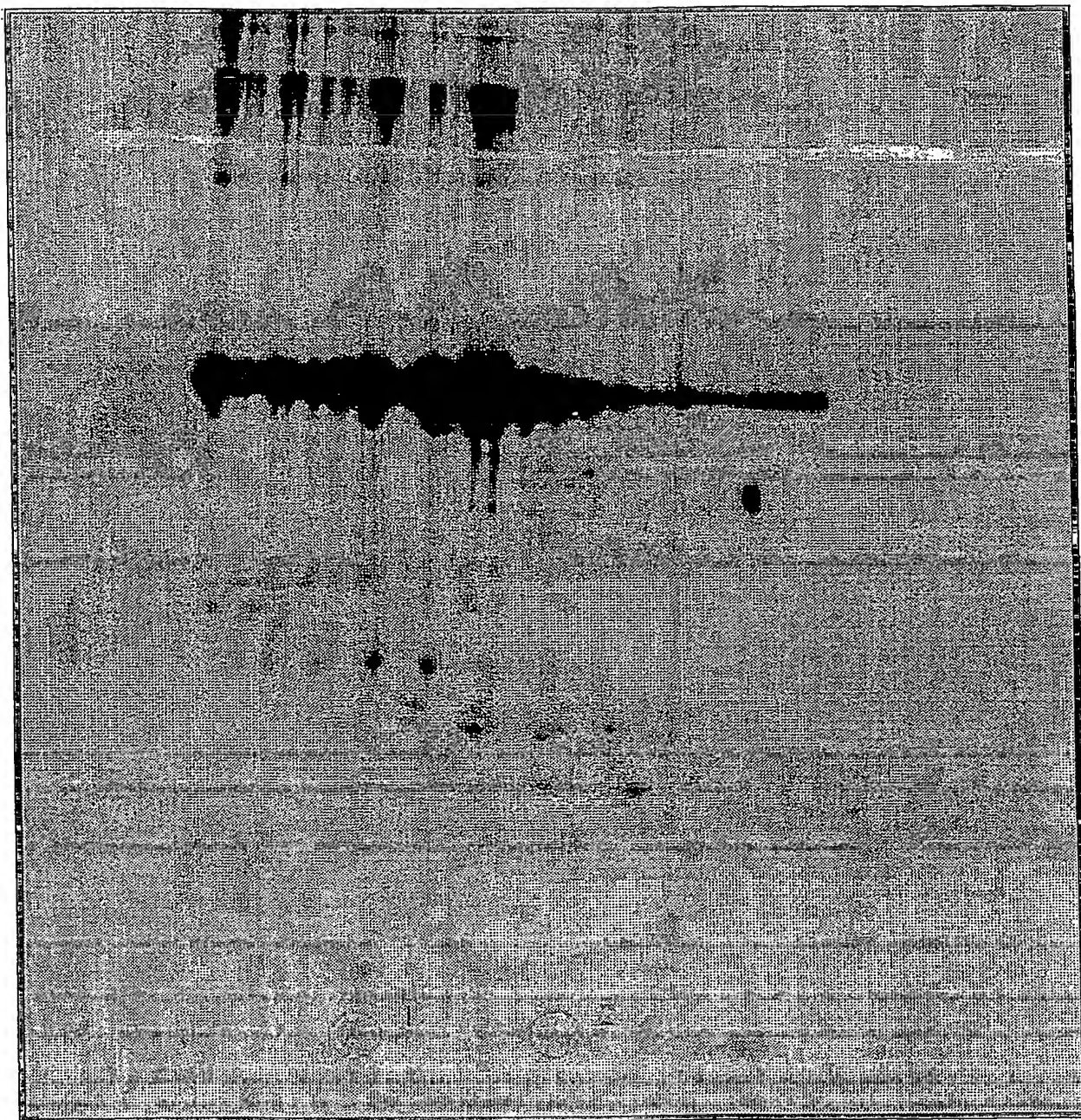
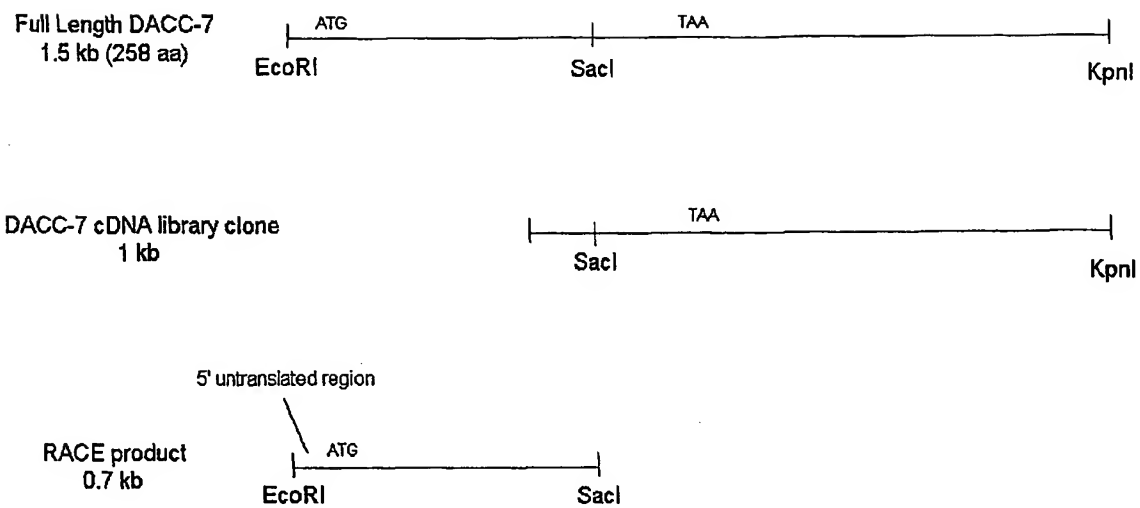


Figure 26

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**Figure 27**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00163

A. CLASSIFICATION OF SUBJECT MATTER														
Int. Cl. ⁷ : C07K 14/435, 14/78; A61K 38/17, 38/39														
According to International Patent Classification (IPC) or to both national classification and IPC														
B. FIELDS SEARCHED														
Minimum documentation searched (classification system followed by classification symbols)														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) GenPept, TREMBL, SWISS-PROT, PIR														
C. DOCUMENTS CONSIDERED TO BE RELEVANT														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
P,X	STRAUSBERG, R.: GenPept Entry, Accession No. AAH 15349, submitted 1 October 2001.	50-52, 61, 62												
X	ELIMA, K. ET AL.: Medline Abstract 8806771; Nucleic Acids Res. 15(22), 9499-9504 (1987), Accession No. CAA29604.	53, 61, 62												
X	CARNINCI, P. ET AL.: Medline Abstract 99279253; Meth. Enzymol. 303, 19-44 (1999), Accession No. BAB28188.	54, 61, 62												
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex														
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Date of the actual completion of the international search 19 April 2002		Date of mailing of the international search report 06 MAY 2002												
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00163

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KIKUNO, R. ET AL.: Medline Abstract 99397452; DNA Res. 6(3), 197-205 (1999), Accession No. BAA83027.	56, 61, 62
X	SWISS-PROT Entry, Accession No. HSBX_HUMAN STANDARD, Submitted October 1997.	57, 61, 62
P,X	VALKILLA, M. ET AL.: Medline Abstract 21451029; Matrix Biol. 20(5-6), 357-366 (2001), Accession No. AAL13166	58, 61, 62
X	TROMP, G.: Medline Abstract 89025644; Biochem. J. 253(3), 919-922 (1988), Accession No. AAB94054.	59, 61, 62